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Space Station Biological Research Project—Reference Experiment Book

Catherine Johnson and Charles Wade, Editors

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Ames Research Center
Moffett Field, California 94035-1000

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ACKNOWLEDGEMENTS

The experiments included in this report are the results of the efforts of the Gravitational Biology Facility Science Working Group who provided many of the experiments themselves as well as soliciting others from their colleagues. These experiments provide guidelines for the development of hardware to be used on International Space Station for life sciences research and provide insight into the type of specimen manipulation and preservation techniques which will be required. They provide an essential measuring stick for development of the hardware and software.

Members of the working group Cell Biology Discipline team were: Drs. Terry Johnson, Joseph F. Albright, Bruce Umminger, Peggy Whitson, William P. Weismann; of the Developmental Biology Discipline team: Drs. Patricia Hester, Dora Hayes, Martin Morton, Carey Phillips, Gerald Schatten, Richard Wassersug; and of the Plant Biology Discipline team: Drs. Norman Lewis, Arnold J. Bloom, Gerald Deitzer, Lewis Feldman, Fred Sack, and Abraham D. Krikorian. Their efforts in developing the experiments is greatly valued and appreciated. We want to thank them for this support.

Special thanks must also go to Dr. Charles A. Wade, the Project Scientist for the Gravitational Biology Facility and to Ms. Kellie McKeown, the Project Manager in the formative phases of the Gravitational Biology Facility who organized the many workshops to define the science requirements for the specimen habitats which will house the specimens to be utilized in these reference experiments. The Lockheed-Martin contractor science staff, especially Ms. Carolyn Ronzano, are primarily responsible for the production of this report.

John J. Givens, Project Manager
Space Station Biological Research Project
NASA Ames Research Center

INTRODUCTION

DOCUMENT PURPOSE

The Space Station Biological Research Project (SSBRP), which is the combined efforts of the Centrifuge Facility (CF) and the Gravitational Biology Facility (GBF), is responsible for the development of life sciences hardware to be used on the International Space Station to support cell, developmental, and plant biology research. The SSBRP Reference Experiment Book was developed to use as a tool for guiding this development effort. The reference experiments characterize the research interests of the international scientific community and serve to identify the hardware capabilities and support equipment needed to support such research. The reference experiments also serve as a tool for understanding the operational aspects of conducting research on-board the Space Station. This material was generated by the science community by way of their responses to reference experiment solicitation packages sent to them by SSBRP scientists. The solicitation process was executed in two phases. The first phase was completed in February of 1992 and the second phase completed in November of 1995. Representing these phases, the document is subdivided into a Section I and a Section II.

The reference experiments contained in this document are only representative microgravity experiments. They are not intended to define actual flight experiments. Ground and flight experiments will be selected through the formal NASA Research Announcement (NRA) and Announcement of Opportunity (AO) experiment solicitation, review, and selection process.

SECTION I

Section I of the Reference Experiment Book was developed in 1992 as part of the process of establishing the scientific foundation for the Gravitational Biology Facility (GBF), which at that time operated as an independent entity. Reference experiment solicitation packages were distributed by the GBF Science Working Group (SWG) members and the GBF Project Scientists to their colleagues in the science community. The range and diversity of the reference experiments was, therefore, dependent to a large extent on the diversity of the SWG. Through the efforts of the SWG and the GBF Project Scientists, solicitation packages were distributed to various research institutions throughout the United States, and in Canada and Russia. In all, more than 220 solicitation packages were distributed. The reference experiments were used by the GBF Project and the GBF Science Working Group to develop an initial list of on-orbit hardware capabilities required to conduct gravitational biology research in the disciplines of cell, developmental, and plant biology.

SECTION II

Section II of the Reference Experiment Book was developed in November 1995. The Space Station Biological Research Project (SSBRP) was formed in January 1995 as a result of the Centrifuge Facility (CF) and Gravitational Biology Facility (GBF) merging to form one project. Since the original solicitation for reference experiments in 1992, much work had been done to define feasible life science hardware capabilities. Feasibility during this period was based largely on budget and current technology available. With this knowledge of hardware capabilities, the type of research which was going to be achievable on-board Space Station was clarified and it provided SSBRP with a more focused vision as compared to the broad vision of the earlier GBF project. This new focus highlighted a need for reference experiments that were more aligned with the direction SSBRP was heading. Therefore a re-solicitation for reference experiments was sent out. These solicitation packages were more specific to current hardware capabilities and the appropriate specimens for such capabilities. The SSBRP solicitation also expanded beyond the limited audience of a SWG network by using the World Wide Web to reach a larger, more diverse, international audience. The result was reference experiments that were more relevant to the project and more appropriate for mission scenarios in support of current hardware planning.

CELL, DEVELOPMENTAL & PLANT DISCIPLINE RATIONALES

The following excerpts from the NASA Life and Biomedical Sciences and Applications Division Space Biology Discipline Plans describe the scientific rationale for each of the three research areas that the GBF supports.

Cell Biology Discipline Plan

"There are four main reasons for studying gravitational effects on cells: (1) to gain fundamental knowledge of the potential influences of the absence of gravity on the cellular functions of both plant and animal cells; (2) to relate the cellular activities, altered under gravity unloading conditions, to a better understanding of events on Earth--in unit gravity--that are associated with the regulation of cell proliferation, gene action, differentiation, development, etc.; (3) to exploit altered cellular functions that occur in the microgravity environment to generate biotechnology and products that will improve the quality of life; and (4) to provide accurate projections of those long-term influences on cellular functions that may impede future space exploration."

Developmental Biology Discipline Plan

"Ground studies together with flight studies show that altered gravity profoundly affects development in several non-vertebrate animal species. These observations indicate that events including gametogenesis, fertilization, and embryogenesis can in principle, respond to changes in gravity. However, none of these studies examined serial generations of propagating organisms, and no animal organism has traversed a complete life cycle (egg to egg) during sustained altered gravity conditions. Consequently, we do not yet know for any animal organism which of these responses are (1) reversible over the short or long-term and (2) compatible with life over serial generations, and might over time lead to (3) heritable changes in the genome and hence behavior and appearance (phenotype) of the organism."

Plant Biology Program Plan

"While the SBPP (Space Biology Plant Program) is focused primarily on enhancing fundamental science--understanding the effects of gravity on plants is a scientific question of inherent, substantial, scientific value--it is intended to also provide knowledge that could contribute to the development of bioregenerative life support systems for long term human habitation of space, and that could lead to better utilization of plant resources on earth. The two goals of the SSBRP, therefore, are to achieve a fundamental scientific understanding of the effects of gravity on plants, and to provide basic knowledge of plant development and physiology through experimentation in the microgravity environment of space flight that can contribute to the utilization of plants in space and on earth."

AREAS OF RESEARCH SOLICITED

The guidance for the areas of research solicited was taken directly from the NASA Space Biology Program's Cell, Developmental, and Plant Biology Discipline Plans. Each of these plans includes a series of critical scientific questions which, if answered, lead to the accomplishment of the discipline's goals and objectives. The general sub-categories of the questions posed by each of the disciplines are summarized here. The complete list of the critical questions is provided in Appendix A.

Critical Question Sub-Categories

A shorthand letter notation has been assigned to each discipline and its critical question sub-categories, as shown in parentheses.

(C) Cell Biology

- (CA) Gravity Sensing/Response
- (CB) Transduction/Response
- (CC) Other

(D) Developmental Biology

- (DA) Early Developmental Events
- (DB) Later Developmental Events
- (DC) Parturition/Postnatal
- (DD) Multigenerational Studies
- (DE) Behavior/Circadian Rhythms
- (DF) Aging
- (DG) Other

(P) Plant Biology

- (PA) Gravity Perception/Transduction/Response
- (PB) Reproduction; Development
- (PC) Metabolism/Transport
- (PD) Communities/Microecology

HOW TO USE THIS BOOK

FOR SECTION I

To locate experiments:

By General Research Topic:

The reference experiments are organized by discipline (Cell Biology, Developmental Biology and Plant Biology) and gravitational biology critical question sub-category, as defined on page 1-4. Use the Experiment Index, which starts on page 3-1, to find the reference experiments in your area of interest. For ease of use, the relevant discipline and critical question sub-category is printed in the bottom right corner of each page of the reference experiment descriptions.

By Critical Research Question:

The critical questions taken from the NASA Space Biology Program's Cell, Developmental and Plant Biology Discipline Plans are printed in Appendix A, pages A-3, 4; A-6, 7; and A-10, 11 respectively. Use the matrices in Appendix A to identify the experiments that address a particular critical question. Experiments are identified in the matrices by their Experiment Identification Codes. Use of the matrices is described in Appendix A, page A-2. The critical questions addressed by each experiment are also identified in the reference experiment descriptions.

By Experiment Identification Code:

Experiment identification codes are provided for reference and are explained in the Experiment Index, page 3-1. The first letter of the code always identifies the discipline: C = cell, D = Developmental, P = Plant. The second letter categorizes each experiment according to gravitational biology critical question area. The final letter makes each code unique. A legend is also provided at the beginning of each reference experiment's description to explain its specific code. To facilitate location of experiments, the experiment identification codes are printed at the beginning of each experiment description and in the top right corner of each right hand experiment description page.

By Hardware Capability Requirements:

The GBF Hardware Capabilities List, established by the GBF SWG in July 1992, is provided in Appendix D. Use the matrices in Appendix D to identify all of the experiments that require a particular hardware capability. Experiments are identified in the matrices by their Experiment Identification Codes. A list of hardware capabilities required by each reference experiment is also provided in the reference experiment descriptions.

By Experiment Duration:

Use the matrices in Appendix E to identify the reference experiments in each experiment duration category. Experiments are identified in the matrices by their Experiment Identification Codes. Experiment durations are also stated in each reference experiment description.

By Specimen Type:

Appendix F lists all the specimens proposed for study by the reference experiments in table form. Use these tables to identify all the experiments that involve a particular specimen type. Experiments are identified in the list by their Experiment Identification Codes. Information about the experimental specimens required by each experiment is also provided in the reference experiment descriptions.

By Author:

Appendix G identifies all of the authors that contributed to Section I of this document. Experiment identification codes are used to identify the contributions of each author.

FOR SECTION II

To locate experiments:

By General Research Topic:

The reference experiments are organized by discipline (Cell Biology, Developmental Biology and Plant Biology) and gravitational biology critical question sub-category, as defined on page 1-4. Use the Experiment Index, which starts on page 6-1, to find the reference experiments in your area of interest. For ease of use, the relevant discipline and critical question sub-category is printed in the bottom right corner of each page of the reference experiment descriptions.

By Experiment Identification Code:

Experiment identification codes are provided for reference and are explained in the Experiment Index, page 6-1. The first letter of the code always identifies the discipline: C = cell, D = Developmental, P = Plant. The second letter categorizes each experiment according to gravitational biology critical question area. The final letter makes each code unique. The reference experiment codes in section II are followed by (2). A legend is also provided at the beginning of each reference experiment's description to explain its specific code. To facilitate location of experiments, the experiment identification codes are printed at the beginning of each experiment description and in the top right corner of each right hand experiment description page.

By Author:

Appendix K identifies all of the authors that contributed to Section II of this document. Experiment identification codes are used to identify the contributions of each author.

SECTION I

SECTION I DEVELOPMENT

GENERAL PHILOSOPHY

The philosophy of the GBF Project and Science Working Group in soliciting reference experiments was to involve a large and varied group of representatives from the science community, and to obtain as many experiments as possible. The Project's goal was to obtain a minimum of 100 reference experiments to ensure that the hardware developed by the project supported as broad a range of gravitational biology research as possible.

EXPERIMENT SOLICITATION

The experiment development process was initiated in February, 1992. Reference experiment solicitation packages were distributed by the GBF Science Working Group (SWG) members and the GBF Project Scientists to their colleagues in the science community. The range and diversity of the reference experiments was, therefore, dependent to a large extent on the diversity of the SWG. The SWG included members from each of the Cell, Developmental, and Plant Biology disciplines. Within each of these disciplines, at least one member a) had space flight experience, b) had not previously been involved with NASA, c) had experience with research in extreme environments (e.g. high altitudes), and d) was a NASA scientist. (A list of the SWG members is included in Appendix B.)

SOLICITATION PACKAGE

The reference experiment solicitation package included a list of critical space biology research topics derived from the Cell, Developmental and Plant Space Biology Discipline Plans; a list of SWG members; a reference experiment template to guide the scientists in preparing their experiment descriptions; and three sample experiments to show the desired detail and content. A complete solicitation package is included in Appendix C.

SPACE STATION FLIGHT OPPORTUNITIES

The scientists designed their experiments around three different Space Station flight opportunities. The flight opportunity descriptions below were provided in the solicitation package. (Scientists were asked to place emphasis on experiments which could be conducted during the early flight opportunities (Phase 1 and 2).)

Phase 1: Utilization Flights (Spacelab class missions) - This phase provides flight opportunities similar to Spacelab missions with a total flight time of 16 days (approximately 11 days can be used for science payload operations on orbit). The Space Shuttle will be docked at the Space Station and members of the astronaut crew will be available to perform basic experiment procedures including specimen sampling and fixation for ground-based analysis.

Phase 2: Human Tended Capability (Spacelab class missions with extended unmanned durations) - This phase is similar to Phase 1 Missions except the science payload can be left unattended on board the Space Station for 30 - 180 days after the Space Shuttle returns to Earth. This mission requires autonomous science experiments that do not require on-orbit crew manipulation.

Phase 3: Permanent Human Presence Capability (Space Station missions) - This is the most sophisticated stage of the Space Station in which the crew will be permanently on orbit and experiment durations are not limited. Research facility capabilities available on orbit will be more diverse and complex. Specimen handling and analysis will be able to be performed on-orbit.

HARDWARE CAPABILITIES LIST DEVELOPMENT

The development of the GBF hardware capabilities list was based entirely on hardware needs identified by the reference experiments. Therefore, prior to developing the list, the SWG reviewed the experiments for their applicability to the Project, linked them to one or more of the critical questions in the NASA Space Biology Program's Discipline Plans, and then identified areas where additional reference experiments were needed. Additional experiments were obtained for those topics that were not addressed. This included selecting nine experiments from the NASA Life Science Research Objectives and Representative Experiments for the Space Station (NASA Technical Memorandum 89445).

The first draft of the GBF hardware capabilities list was developed during the July 1992 meeting of the GBF SWG. The hardware capabilities identified were also prioritized by the GBF SWG based on science research priorities. For the purposes of this document, the hardware is organized into groups and listed in the order of priority established by the GBF SWG as follows: Specimen Habitats, Multi-discipline Equipment, and Preservation and Storage Equipment.

Habitats Both 0g and 1g environments provided on-orbit

Cell Culture Apparatus

Small Plant Habitat

Insect Habitat

Small Aquatic Habitat (Fresh Water)

Medium Aquatic Habitat (Fresh Water)

Avian Egg Incubator

Rodent Birthing Habitat

Small Aquatic Habitat (Salt Water)

Large Plant Habitat

Medium Aquatic Habitat (Salt Water)

Rodent Breeding Habitat

Avian Hatchling Habitat

Rodent Rearing Habitat

Rodent Weanling to Adult Habitat

Avian Adult Habitat

Large Aquatic Habitat

Multi-discipline Equipment

Data Storage
Fluid Handling Tools
Compound Microscope
Work Area
Dissection Equipment
Dissecting Microscope
Temperature Controlled Chamber
Temperature Controlled Laboratory Centrifuge
Radioisotope Handling Equipment
Digital Multimeter
Ion Selective Electrodes
Spectrophotometer
Mass Measurement Device
Electrophysiology Measuring Equipment
Micromanipulation Device
Luminometer

Preservation and Storage Equipment

Snap/Quick Freezer
-70°C Freezer
Chemical Fixation Capability
Refrigeration (4°C)
-196°C Freezer
-20°C Freezer
Freeze Dryer
Ambient Storage

A matrix identifying the GBF hardware capabilities required by each experiment is included in Appendix D. Additional experiment unique equipment not provided by the Project is required for some of the reference experiments.

REFERENCE EXPERIMENT ORGANIZATION

The author's hypothesis, scientific rationale, general approach and hardware needs are described for each reference experiment. In addition, a section is included in each experiment to indicate which items from the GBF hardware capabilities list are required to conduct each experiment.

Experiment Identification Codes were developed which identify the subcategories of the critical questions posed by the Discipline Plans that are addressed by each experiment. Identification codes were assigned to the experiments based on the primary critical question subcategory addressed by the experiment. Experiments linked to more than one sub-category are cross referenced in the Experiment Index.

DOCUMENT SUMMARY

A total of 134 experiments (37 Cell, 59 Developmental, and 38 Plant) were submitted by 90 different contributors. An author list is provided in Appendix G. Specimens types include cell culture, algae and higher plants, insects, amphibians, aquatics, rodents, and avians.

All research areas noted in the Discipline Plans were addressed by at least one reference experiment. Fifty seven of the total of 67 critical questions were addressed specifically.

Approximately two thirds of the experiments (75% of the cell, 50% of the developmental and 75% of the plant experiments) were designated for the Space Station Phase 1 flight opportunities, and the other third were recommended for Phases 2 and 3. One quarter of the experiments designated for Phase 1 also included options for the later flight opportunities.

REFERENCE EXPERIMENT INDEX

The reference experiments are organized by discipline (Cell Biology, Developmental Biology and Plant Biology) and gravitational biology critical question sub-category.

Each reference experiment has been assigned a three letter code, e.g. CA-a. The first two letters indicate the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The third letter makes the notation unique by indicating where the experiment is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments. CA-b is the second of the Cell Biology Gravity Sensing/Response experiments, etc.

The experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page

Critical Question Sub-categories

(C) Cell Biology

- (CA) Gravity Sensing/Response**
- (CB) Transduction/Response**
- (CC) Other**

(D) Developmental Biology

- (DA) Early Developmental Events**
- (DB) Later Developmental Events**
- (DC) Parturition/Postnatal Growth**
- (DD) Multigenerational Studies**
- (DE) Behavior/Circadian Rhythms**
- (DF) Aging**
- (DG) Other**

(P) Plant Biology

- (PA) Gravity Perception/Transduction/Response**
- (PB) Reproduction/Development**
- (PC) Metabolism/Transport**
- (PD) Communities/Microecology**

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GRAVITATIONAL BIOLOGY FACILITY
REFERENCE EXPERIMENTS

Experiment Identification Code: CA-a C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. N.H. Mendelson & Dr. J.O. Kessler

Department of Molecular and Cellular Biology
LSS Building
University of Arizona
Tucson, AZ 85721

Critical Question(s) Addressed:

CA-2, CB-3 (See p. A-3)

3. Experiment Title

Bacterial Adaptations to Microgravity

4. Purpose/Hypothesis

To determine whether growth in microgravity leads to:

- 1) adaptations that influence cell signaling for regulation of gene expression; and
- 2) adaptations that influence the self-assembly of multicellular structures (macrofibers)

5. Scientific Rationale/Rationale for Microgravity

Cell signaling via diffusible molecules that govern the expression of genes in bacterial colonies has been characterized in normal gravity. Both spatial and temporal aspects of colony growth and cell responsiveness are involved. The system can serve as a model therefore to assess whether microgravity environments disrupt or alter complex processes in multicellularity pertaining to gene regulation. Similarly the biomechanics of macrofiber self-assembly in normal gravity has been shown as an ideal model for study of biomechanical aspects of morphogenesis, and too therefore can be used to assess the influences of long-term microgravity on biomechanics of development.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 (90 days)

B. General Approach Description:

Prior to the flight experiment, ground experiments will be concerned with developing the growth protocols for both signalling and macrofiber experiments, and with data collection and control of data all using *Bacillus subtilis* systems previously studied. The signaling experiments involve growth of colonies on agar blocks of various dimensions and geometries and examining gene expression using X-gal and the *lacZ* reporter system. The macrofiber experiments will be done using fluid cultures at 20°C where right-or left handed structures are produced depending upon the bacterial strain, at the rate of about one fiber cycle per 24 hrs. Self assembly is studied by time-lapse microcinematography. The experiment will include on-orbit 1-g controls.

C. Number and Type of Specimen:

Four bacterial strains will be used: 5:7 and F2b for signalling experiments and FJ7 and RHX for macrofiber morphogenesis. Duplicate cultures will be used in all experiments.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Preparation of agar blocks; culture transfer and inoculation; photographic and video imaging of cell signalling experiments; microscopic imaging (fed to CCD and recorded with time-lapse VCR) of macrofiber experiments.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Pattern analysis from data recorded on film. Deviations in temporal or spatial order from controls for cell signaling experiments. Changes in parameters such as twist state, helix hand, folding threshold, or folding route from controls for macrofiber experiments.

F. Experiment Controls:

In-flight: 1-g in-flight controls.

Ground: Ground-based controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Sterile culture transfer facility. Incubators (temperature controlled from 20 to 48°C). Microscopes with low power objectives (4 to 10X). 35mm camera and CCD video camera. Time lapse VHS recorder. Light sources to visualize blue color development as a result of X-gal hydrolysis. Cutting jig for production of sterile agar blocks of uniform size.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, (temperature controlled chamber), work area, fluid handling tools, compound microscope, data storage, refrigeration (4°C).

Experiment Identification Code: CA-b C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Beth A. Montelone

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CA-2 (See p. A-3)

3. Experiment Title

Chromosome Segregation in Microgravity

4. Purpose/Hypothesis

The precise assortment of chromosomes into daughter cells in every mitosis is accomplished by spindle fibers. Alterations in their structure in microgravity could lead to increased incidence of nondisjunction. We will genetically measure nondisjunction frequency in the model eukaryote *Saccharomyces cerevisiae*.

5. Scientific Rationale/Rationale for Microgravity

Cytoskeletal elements seem to be sensitive to gravitational state and may govern cell responses. Chromosome partitioning in cell division should be a particularly good indicator of the functioning of the cytoskeleton in response to altered gravitation. This will allow us to ask basic questions pertaining to long term growth of organisms in space.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Diploid yeast cells bearing appropriate genetic markers will be grown in culture from small inocula to saturation. Cultures will be stored at 4°C for postflight analysis.

C. Number and Type of Specimen:

Laboratory yeast strains with different genetic backgrounds will be used. Duplicate cultures of four different strains will be grown (8 total).

D. Measurements/Sample Handling:

Preflight: Prepare strains.

In-flight: Start cell cultures; refrigerate when grown to appropriate density.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Plate cultures and replica plate colonies for genetic analysis; score plates.

F. Experiment Controls:

In-flight: None.

Ground: Parallel cultures to flight samples.

7. Hardware Requirements

A. Minimum on Orbit Requirements

30° refrigerated incubator with agitation (not CO₂); sterile sampling environment (hood); 4°C refrigerator.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, work area, refrigeration (4°C), fluid handling tools.

Experiment Identification Code: CA-c C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. James E. Urban

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CA-2, CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on the Induction of Nitrogen-fixing Bacteroids in *Rhizobium* sp.

4. Purpose/Hypothesis

Microgravity will enhance the binding by *Rhizobium* cells of molecules which stimulate rhizobia to become bacteroids.

5. Scientific Rationale/Rationale for Microgravity

Experiments conducted during microgravity episodes created by parabolic flight in the NASA 930 aircraft show that rhizobial cells bind more bacteroid-inducing molecules in reduced gravity. The variability in quality of microgravity and its short duration require prolonged exposure to constant and quality microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

At intervals cells being held in microgravity will be exposed to inducer for 1 hour then fixed in formaldehyde. The amount of inducer bound to cells will be determined postflight.

C. Number and Type of Specimen:

Rhizobium bacterial cells and the inducing acetyl salicylic acid will be used. Approximately 4 replicate samples will be collected each day (4 x 15 = 60 total samples).

D. Measurements/Sample Handling:

Preflight: Prepare cells and inducer for mixing, prepare formaldehyde fixative.

In-flight: Each day replicates of cells and inducer will be mixed. After one hour of incubation, samples will be fixed with formaldehyde.

Postflight: Assay amount of inducer bound.

E. Specific Sample Analysis:

In-flight: None

Postflight: Collect cells by centrifugation, quantitate acetyl salicylic acid binding colorimetrically.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Ground (1-g) controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

30°C shaking incubator in a 1-atm environment, sterile cell handling, formaldehyde adding environment.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, refrigeration (4°C).

Experiment Identification Code: CA-d C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Gabrielle Meeker

Space Station Biological Research Project
NASA Ames Research Center
Moffett Field, CA

Critical Question(s) Addressed:

CA-2, CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on the Induction of Nitrogen-fixing Bacteroids in *Rhizobium* sp. Part II - The Capability of Induced *Rhizobium* to Attach to Plant Cells and Form Rhizoids in MicroGravity

4. Purpose/Hypothesis

Microgravity will enhance the ability of *Rhizobium* to attach to plant cells and form rhizoids.

5. Scientific Rationale/Rationale for Microgravity

The ability of *Rhizobium* to fix Nitrogen for plants to use depends upon *Rhizobium*'s ability to bind to molecules produced by the plant, followed by attachment/invasion with plant cells. Parabolic flights have shown that rhizobial cells bind more bacterioid-inducing molecules in reduced gravity. If corroborated by prolonged exposure to constant microgravity (Part I of this series), then the ability of the induced microbes to attach to plant cells and form rhizoids should then be tested. The ability of the plant to produce *Rhizobium* attracting molecules can be tested concurrently.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Seedlings will be started in culture vessels on agar and inoculated with *Rhizobium* pre-flight. At intervals, culture vessel contents will be frozen for post-flight analysis on the ground.

C. Number and Type of Specimen:

Rhizobium bacterial cells. Approximately 4 replicates of the vessel contents will be collected per sampling time (4 x 15 = 60 total samples).

D. Measurements/Sample Handling:

Preflight: Prepare seedlings, and *Rhizobium*.

In-flight: Contents of the whole culture vessel will be frozen periodically.

Postflight: Assays will be done to determine the amount of inducer produced and the extent of nodule initiation.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Separate plants from the agar and dissect out roots. Assess nodule formation. Assay the agar for inducer.

F. Experiment Controls:

In-flight: One experimental set-up without *Rhizobium* should be used to assay production of inducer. 1-g control should also be done.

Ground:
Ground (1-g) controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Incubator, freezer safe culture vessels prepared with specimen, -80°C freezer, on-orbit transporter for specimen transfer to the freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Incubator, -80°C freezer, data storage

Experiment Identification Code: CA-e C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Rose Grymes

NASA Ames Research Center
MS 239-11
Moffett Field, CA 94035

Critical Question(s) Addressed:

CA-2, CB-2, DF-1 (See p. A-3, A-7)

3. Experiment Title

Cytoskeletal Element Changes in Normal and Senescent Cells Exposed to Microgravity

4. Purpose/Hypothesis

To observe and record the microgravity related re-organization of the cytoskeleton, and to compare the response of normal and aged cells.

5. Scientific Rationale/Rationale for Microgravity

Previous spaceflight experiments have suggested parallels between the behavior and metabolism of aged or senescent cells with those of cells exposed to microgravity. The role of the cytoskeleton in providing structure and tensional integrity to cells may be uniquely affected by the absence of 1-g in space.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Normal dermal fibroblasts of several different chronologic ages, similar cells made senescent *in vitro*, and cells of the genetic background Werner's syndrome (an accelerated aging disease) will be cultured in flight in both microgravity and 1-g conditions. Cells will be stimulated with a mitogen known to induce cytoskeletal re-arrangements and translocation of important second messengers involved in signal transduction. Cells will be fixed at short times following such treatments.

C. Number and Type of Specimen:

Dermal fibroblasts are attachment dependent cells. Fetal, young adult, older adult, aged, *in vitro* senescent, and Werner's syndrome cells will be studied (6 cell types). Replicates will be examined with and without mitogen stimulation (X2), and flight experiments will require on-board centrifuge controls (X2). All conditions and types will be examined in duplicate (X2), for a total of 48 flight specimens.

D. Measurements/Sample Handling:

Preflight: Prepare cell containing chambers, load culture medium, mitogen, and fixative.

In-flight: Mix cells with mitogen/fixative according to schedule.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Fluorescent biprobe and antibody staining of samples, observation and photography using epifluorescent microscope.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, triple mixing chamber or equivalent.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, refrigeration (4°C).

Experiment Identification Code: CA-f C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Valerie E. Martindale

Clinical Investigation Directorate
Wilford Hall Medical Center
Lackland AFB
San Antonio, TX 78236-5300

Critical Question(s) Addressed:

CA-2, CB-3 (See p. A-3)

3. Experiment Title

Alternate Cytoskeletal Gene Expression as an Adaptation to the Microgravity Environment

4. Purpose/Hypothesis

Prolonged exposure to microgravity will induce cells to fine-tune the cytoskeleton to the different environmental demands by alternate expression of the members of the gene families encoding cytoskeletal proteins.

5. Scientific Rationale/Rationale for Microgravity

The cytoskeleton is essential for cell shape, motility, division, and maintenance of internal organization. It is expected to be affected by microgravity conditions, probably at multiple points. Most, if not all, cytoskeletal proteins in eukaryotes are encoded by multiple genes in gene families. Expression of individual members of gene families is regulated developmentally, temporospatially, and in at least some cases in response to environmental demands (e.g. fiber-type switching in skeletal muscle in response to usage pattern). Alternate transcripts can be made from single genes, as well. Alternate gene expression will represent an inherent adaptational capacity used in a fundamental way.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 90 days

B. General Approach Description:

Cells will be cultured in both 0-g and 1-g conditions. Replicate cell samples will be taken at each population doubling for detailed cytoskeletal protein analysis. Protein analysis will be carried out on cytoskeletal extracts, using 2-dimensional gel electrophoresis, transfer to nitrocellulose, protein staining and digital image analysis. Western blotting and mRNA analysis may be performed to follow up on interesting results.

C. Number and Type of Specimen:

A differentiated and a de-differentiated cell line will be used. The number of samplings depends on the rate of population doubling. One sample from each cell line in each condition every 48 hours would be expected. (over 90 days, a total of 180 samples)

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks.

In-flight: Set up cultures, maintain by feeding and splitting, sample every 48 hours, process half of the sample for 2-dimensional gel electrophoresis and store the other half at -196°C .

Postflight: Analyze electrophoresis results for changes over time in both 0-g and 1-g conditions, and for differences between the two. If results indicate, Western blotting for specific proteins and mRNA analysis of specific messages may be performed with the frozen samples.

E. Specific Sample Analysis:

In-flight: Process half of the samples for 2-dimensional gel electrophoresis, and store the other half at -196°C . Perform electrophoresis, transfer of proteins to nitrocellulose, and staining. Photograph or digitally record results. Store blots for postflight analysis.

Postflight: Analyze electrophoresis results, for changes over time in both 0-g and 1-g conditions, and for differences between the two. If results indicate, Western blotting for specific proteins and mRNA analysis of specific messages may be performed with the frozen samples.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Parallel 1-g controls.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Both 0-g and 1-g CO_2 cell incubators; sterile sampling conditions; cell culture maintenance supplies; two dimensional protein electrophoresis and nitrocellulose transfer apparatus; microcentrifuge; high quality digital imaging capabilities, preferably with built in image analysis program; -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, temperature controlled chamber, data storage, work area, fluid handling tools, temperature controlled laboratory centrifuge, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CA-g C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Carey R. Phillips

Department of Biology
Bowdoin College
Brunswick, ME 04011

Critical Question(s) Addressed:

CA-2 (See p. A-3)

3. Experiment Title

Effects of Microgravity on Cell Motility

4. Purpose/Hypothesis

Microgravity will effect the cytoskeletal components involved in cell motility, cell division and cell differentiation.

5. Scientific Rationale/Rationale for Microgravity

Microtubules have been shown to be pressure sensitive. During cell migration and division the microtubule system is in a very dynamic state which may be affected by a microgravity environment. It has also been demonstrated that some species of mRNA need to be physically associated with the cytoskeleton in order to be translated. Therefore, the physical nature of the cytoskeleton may exert subtle effects on the timing and the state of the cell differentiation.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and Phase 2

B. General Approach Description:

Cell cultures would be maintained at microgravity and under various microgravity situations. Cultured embryonic cells (or any other cell types such as fibroblasts) will be examined in flight by a microscope with a computer controlled stage (three dimensional translation) and viewed by video microscopy. Both the stage and the video should be down-linked to a ground based station, or the inflight equipment could be operated by a scientist. A system for incubation of each culture (with controlled environmental conditions) and the changing of specimens to be viewed should be engineered so that regular time points can be recorded. It would be extremely useful if this system were designed such that the cultures could be subsequently fixed for further ground-based, postflight analysis. This same system could be used to study cell-cell interactions of cell aggregation.

C. Number and Type of Specimen:

The more cultures which can be made accessible, the better. This type of experiment could be used for a large variety of specimens.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Observations of cell behavior would be directed from a ground-based station and all data would be down-linked so that radio-controlled manipulations of cultures could be maintained.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Observations of cell behavior.

Postflight: Cytological analyses.

F. Experiment Controls:

In-flight: Controls at various levels of simulated gravity in-flight.

Ground: Ground-based controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Compound microscope with a radio-controlled stage. An interactive computer controlled robotic system for moving samples from the incubation environment to the microscope stage. High quality CCD video equipment. The microscope should have 10x, 40x phase, Nomarski and fluorescence; 4x, 10x, and 20x darkfield for low mag population and migration studies. Cell culture system, CO₂ and O₂ incubator.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, fluid handling tools, work area, compound microscope, data storage, refrigeration (4°C).

Experiment Identification Code: CA-h C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Todd Bergren

BioServe Space Technologies
University of Colorado Campus Box 429
Boulder, CO 80309

Critical Question(s) Addressed:

CA-3, DB-3 (See p. A-3, A-6)

3. Experiment Title

Directed Assembly of Collagen in Microgravity

4. Purpose/Hypothesis

Collagen assembled *in vitro* does not exhibit the organization of *in vivo* collagenous structures. Small forcing functions may be able to align collagen fibers during self assembly.

5. Scientific Rationale/Rationale for Microgravity

Convection flows set up in *in vitro* conditions may overwhelm any forcing function applied to assembling collagen. Convection is damped out in the microgravity environment. In the absence of convective flows, a small forcing function may be able to align collagen fibers during self assembly.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Samples will be allowed to assemble under the influence of forcing functions and will be fixed while in microgravity. Turbidometric measurements will be taken during the assembly process.

C. Number and Type of Specimen:

Three forcing functions (electric current, magnetic field, template) and a control will be used. Four duplicates of four concentrations of collagen will be tested for each of the experimental conditions (64 total samples).

D. Measurements/Sample Handling:

Preflight: Prepare experimental fluids.

In-flight: Initiate and terminate assembly, control forcing functions and data collection, fix samples.

Postflight: Histological sectioning of samples. Mechanical testing of samples.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assembly time courses compared. Sections examined with birefringent microscopy to determine differences in organization. Mechanical properties compared.

F. Experiment Controls:

In-flight: Nondirected samples.

Ground: Same as flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

BioServe's Automated Generic Bioprocessing Apparatus (AGBA) or equivalent automated test facility, data logger/controller, constant power supply.

B. GBF Hardware Capabilities Required (on Orbit)

(Temperature controlled chamber), chemical fixation capability, data storage, fluid handling tools, work area, spectrophotometer, refrigeration (4°C).

Experiment Identification Code: CA-i C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Konrad W. Pollmann

BioServe Space Technologies
University of Colorado Campus Box 429
Boulder, CO 80309

Critical Question(s) Addressed:

CA-3, DB-3 (See p. A-3, A-6)

3. Experiment Title

Assembly of Fibrin Under Non-sedimenting Conditions

4. Purpose/Hypothesis

Macroscopic analysis of biomolecule sedimentation cannot reveal its influence on the molecular assembly process. Absence of the unidirectional gravity field will result in structures whose assembly is dominated by minor forces or superimposed forcing functions.

5. Scientific Rationale/Rationale for Microgravity

To determine the relevant forces governing the assembly of structural biomaterials from molecular monomers. To estimate gravity effects on reacting molecules and molecule clusters.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Mixing of fibrinogen and thrombin solutions will initiate fibrin polymerization. The relative importance of various forces on the assembly will be varied by changing assembly conditions and superimposing external forces. The samples will be fixed on orbit and returned for analysis.

C. Number and Type of Specimen:

Start with 4 duplicate samples for 20 different solutions and external forcing functions (80 total). One fibrinogen solution, thrombin solution and fixative solution for each sample needed.

D. Measurements/Sample Handling:

Preflight: Prepare, load solutions.

In-flight: Initiate assembly, introduce forcing functions (magnetic & electric field, etc.), fix after assembly completion.

Postflight: Analyze molecular and network structure, and macroscopic gel properties (permeability, strength, etc.)

E. Specific Sample Analysis:

In-flight: Monitor turbidity and polarization of transmitted light.

Postflight: Molecular structure: electron microscopy, gel network: histology, stereoscopic laser microscopy; macroscopic properties: materials testing, perfusion measurement.

F. Experiment Controls:

In-flight: None.

Ground: Controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Fluid handling and mixing devices, turbidimeter, (forcing function generators: magnets, electric fields).

B. GBF Hardware Capabilities Required (on Orbit)

(Temperature controlled chamber), chemical fixation capability, fluid handling tools, data storage, work area, spectrophotometer, refrigeration (4°C).

Experiment Identification Code: CA-j C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Konrad W. Pollmann

BioServe Space Technologies
University of Colorado Campus Box 429
Boulder, CO 80309

Critical Question(s) Addressed:

CA-3 (See p. A-3)

3. Experiment Title

Measurements of Diffusion Coefficients for Counter-diffusing Solutes in Complex Solutions

4. Purpose/Hypothesis

Diffusive transport mechanisms in the cytoplasm of living cells cannot be sufficiently approximated by diffusion coefficients measured in homogeneous solutions. Diffusivity measurements under counter-diffusive conditions simulate the environment in the cytoplasm much better, and can then allow estimates of the relative importance of active and diffusive transport in a cell.

5. Scientific Rationale/Rationale for Microgravity

Need to determine diffusion coefficients *in vitro* under counter-diffusive conditions in complex solutions to estimate relative importance of diffuse transport in cells. For many complex solutions, counter-diffusion measurements in 1-g induce density instabilities and convection. Microgravity is needed to eliminate convection while maintaining important experimental parameters.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 (30 days)

B. General Approach Description:

Model solutions for cytoplasmic and/or intracellular fluids will be allowed to counterdiffuse for predetermined time periods in sliding diffusion cells. The experiment will be initiated and terminated automatically during the absence of the crew. The terminated samples will be quenched (rapidly frozen) and returned for postflight analysis of transfer and concentration profiles.

C. Number and Type of Specimen:

400, various solutions of different complexity.

D. Measurements/Sample Handling:

Preflight: Prepare, load solutions.

In-flight: Initiate diffusion, terminate diffusion and quench samples, keep frozen.

Postflight: Unload samples and store at -70°C prior to analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of sample content and concentration profiles in each well by controlled thawing of sample and HPLC.

F. Experiment Controls:

In-flight: None.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Sliding diffusion cell blocks, quenching (quick freezing) device, and -70°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, (temperature controlled chamber), data storage, snap/quick freezer, -70°C freezer, work area, refrigeration (4°C).

Experiment Identification Code: CA-k C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Frederick J. Pearce

Walter Reed Army Medical Center
Walter Reed Army Institute of Research, Bldg. 40
Division of Surgery
Washington, D.C. 20307-5100

Critical Question(s) Addressed:

CA-3, CA-5 (See p. A-3)

3. Experiment Title

Effects of Microgravity on the P50 for Cellular Respiration in Isolated Cells

4. Purpose/Hypothesis

Microgravity will increase the extracellular-mitochondrial oxygen gradients causing an increase in the oxygen concentration required to maintain cellular respiration and mitochondrial oxidative phosphorylation. Large cells with no intracellular myoglobin will be more susceptible to this effect.

5. Scientific Rationale/Rationale for Microgravity

Previous results have shown that the oxygen tension required to maintain cellular respiration and oxidative phosphorylation in isolated cardiac myocytes depends on the absolute rate of oxygen consumption, the size of the cell and the type of available substrate. These factors contribute to variable degrees to the diffusive and convective delivery of oxygen to the mitochondria of the cell. Since convection and diffusion are altered in microgravity environments, a right-ward shift in the relationship of oxygen concentration to oxygen consumption may be expected which would result in a decrease in the oxidative phosphorylation rate at any given oxygen tension and would compromise cellular function. An energy deficit caused by this mechanism could contribute to the muscle wasting observed in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Primary myocytes, L8 skeletal muscle cell line and neuroblastoma cell stock preparations will be introduced into a stirred, temperature controlled cuvette fitted with a fiber optic probe which will deliver excitation light (520 nm) and collect the fluorescent and phosphorescent emitted light (> 630 nm). Oxygen consumption rates will be measured as the oxygen tension in the cuvette falls and the P50 determined at optimized stirring rates. Since stirring rate will affect the extent of the diffusion barrier at the cell surface, several different stirring rates will be studied. Cell suspension will be precipitated in acid and saved for postflight analysis of phosphorylation potential.

- Development of a new technique of accurately measuring oxygen concentration by measurement of oxygen quenched phosphorescence of Pd-coproporphyrin in the range which is limiting to cellular respiration (0-10 mm Hg), now makes this line of investigation feasible.

C. Number and Type of Specimen:

The P50 for each cell type (3) will be determined in quintuplicate (5) at both high (uncoupled) and low (resting) rates of oxygen consumption at 3 different stirring rates. (90 total P50 determinations).

D. Measurements/Sample Handling:

Preflight: Prepare cell stock and Pd-coproporphyrin stock solution.

In-flight: Determine P50 and freeze cell suspensions (-196°C).

Postflight: Precipitate frozen cell suspensions in acid, neutralize and assay for nucleotides.

E. Specific Sample Analysis:

In-flight: P50 determination calculated by onboard-computer or raw data can be downloaded for ground-based analysis important for design of the subsequent experiment.

Postflight: Frozen cells will be acid precipitated and analyzed for ATP, ADP, AMP, Pi, creatine, creatine phosphate, lactate and pyruvate.

F. Experiment Controls:

In-flight: Internally controlled.

Ground: Identical experiment run with the same cells at 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Cells will be maintained in suspension using the Space Tissue Loss Module (STL, Walter Reed Army Institute of Research) and aliquots of cells sampled periodically for P50 determination. A fiber-optic based phosphorimeter controlled by a small microcomputer will be used to acquire and store the raw data and for calculation of PO₂ from the half-life of the phosphorescence decay.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, compound microscope (to measure changes in phosphorescence), data storage, work area, fluid handling tools, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CA-1 C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Christopher C. Field

Southwest Missouri University
Department of Biomedical Science
901 S. National Avenue
Springfield, MO. 65804-0094

Critical Question(s) Addressed:

CA-3, CB-3 (See p. A-3)

3. Experiment Title

Macromolecular Assembly in Microgravity

4. Purpose/Hypothesis

Complex macromolecular interactions will be disrupted in microgravity.

5. Scientific Rationale/Rationale for Microgravity

The synthesis and assembly of a virus involves many independent cellular activities that must occur in the correct time and space for formation of a functional virus. Because the reaction is initiated at the time of viral attachment, the onset of the reactions to be studied can be controlled. Secondly, the steps in viral synthesis are well characterized and open to analysis of this most simple biological entity. Third, because the virus depends on the host for formation, an alteration of the host will affect the viral production, thus amplifying the change in the host.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (28 days)

B. General Approach Description:

Traditionally utilized and susceptible cell cultures will be infected with appropriate viruses: Vaccinia (complex virus), Sindbis (simple, enveloped virus). Polio (vaccine strain, simple naked virus), and SV40 (transforming, naked virus). Cultures will be infected with the appropriate viruses on day one of the flight. Microscopic analysis for cytopathic effect and sample collection will be done daily, if microscopic analysis is available.

C. Number and Type of Specimen:

Four samples of each viral type will be analyzed.

D. Measurements/Sample Handling:

Preflight: Initiate growth of cell lines.

In-flight: Inoculate cells analyze microscopically, collect and fix or freeze samples.

Postflight: Collect cultures and analyze for virus production.

E. Specific Sample Analysis:

In-flight: Microscopy.

Postflight: Quantitate viruses, analyze cells for CFE and viral nucleic acids (DNA and RNA) and proteins by gels, blots, and probes, and transformation (SV40).

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, sterile sampling/set up environment (laminar flow hood), or Kearney Orbital Growth Chambers, -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, work area, fluid handling tools, snap/quick freezer, -196°C freezer, refrigeration (4°C), compound microscope, data storage.

Experiment Identification Code: CA-m C = cell; A = gravity sensing/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Gregory A. Nelson

Space Biological Sciences Group, MS 89-2
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, CA 91109

Critical Question(s) Addressed:

CA-3 (See p. A-3)

3. Experiment Title

Viral Capsid Assembly in Microgravity

4. Purpose/Hypothesis

The complex processes of slow collision, recognition of multiple corresponding protein surfaces, conformational changes and binding of viral capsid components will proceed with true diffusion limited kinetics in microgravity. The viral assembly kinetic constants can be measured under flow conditions such as obtained intracellularly.

5. Scientific Rationale/Rationale for Microgravity

Viral self-assembly can be brought about in *in vitro* at low efficiency but convection, concentration gradients, and mixing artifacts prevent acquisition of kinetic data under the truly diffusion-limited conditions as exist inside a bacterial cell. By conducting an *in vitro* assembly in microgravity true diffusion-limited conditions are generated and assembly as a function of component concentration and time can be accurately measured. This process is biologically significant as it is a model of self assembly for complex structures in general and is relevant to microgravity science as an example of self-assembly of a molecular machine by processes more complex than the phase change of crystallization.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Head and tail defective mutants of the *E. coli* bacteriophage P2 and T4 are used to produce incompletely assembled heads and tail components which can be mixed sequentially to produce complete, active virus particles (molecular machines which act like syringes to inject DNA into a cell). The natural process requires complex recognition, allosteric changes and binding processes which can be accomplished *in vitro* by mixing the components in the proper stoichiometric ratios. Rate constants for the processes and completeness of the reaction are measured for various time intervals at controlled temperatures and concentrations followed by stopping the reactions by addition of inhibitors and/or rapid cooling. To analyze successful assembly, susceptible *E. coli* cultures are challenged with the mixtures and plaque analysis determines successful viral infection. Electron microscopy of mixtures is used to characterize partial assembly.

C. Number and Type of Specimen:

Bacteriophage P2 and T4 head and tail components are held in 50 to 500 microliter compartments in a mixing device. 10 time points in triplicate for each of 4 phage component combinations are likely (120 total) each with about 10^8 particles.

D. Measurements/Sample Handling:

- Preflight: Purified phage components are prepared from infected bacterial cultures and assayed for activity and concentration. Appropriate concentrations of components are loaded into sample containers and stowed.
- In-flight: Samples in hardware unstowed and placed in incubator. Mixing of samples according to predetermined timeline is performed resulting in a series of reactions as functions of time and component characteristic. Completed reactions are stowed at low temperature.
- Postflight: Reaction mixtures are divided into aliquots for electron microscopy and infection of *E. coli* cultures over a range of titers. The number of plaques are quantified to measure successful assembly. Electron microscopy is performed as needed to characterize partial or inappropriate assembly.

E. Specific Sample Analysis:

- In-flight: Sample analysis is postponed until landing.
- Postflight: Pour-plate techniques for generating viral plaques are used to quantify the number of active virus particles assembled in microgravity.

F. Experiment Controls:

- In-flight: In-flight centrifuge control should be performed with same lot of materials. The expected difference is the presence of convection cells but no significant isothermal settling.
- Ground: Parallel ground controls with same lots should be performed.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Incubation at 37°C or 20°C with and without 1-g centrifuge. Crew or automatically activated sample mixing hardware is needed with stow/unstow in cold at -20°C.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, fluid handling tools, work area, -20°C freezer.

Experiment Identification Code: CB-a C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Marian L. Lewis

Science Bldg., Room 360
University of Alabama
Huntsville, AL 35899

Critical Question(s) Addressed:

CA-1, CA-2, CB-1, CB-3 (See p. A-3)

3. Experiment Title

Signal Transduction and Gene Expression in Gravity-sensitive Cells

4. Purpose/Hypothesis

Mechanisms resulting in the failure of certain precursor cells to differentiate in microgravity may be due to low-g effects on signal transduction pathways and altered expression of specific genes.

5. Scientific Rationale/Rationale for Microgravity

Studies with hematopoietic precursors and epithelial cells in response to differentiation mediators suggest that signal transduction involving protein kinase C and the cytoskeleton may be affected in gravity-sensitive cells. This study will provide cell-level basic information on gene expression and the role of the cytoskeleton in low-g "sensing".

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be mixed with mediators in 1-g, and in low-g and a 1-g centrifuge on orbit. Duplicate samples will be fixed after 10 minutes, 2 and 12 hours and at daily intervals thereafter and stored at 20°C for evaluation after flight.

C. Number and Type of Specimen:

Lymphocytes and an attachment-dependent epithelial cell line will be evaluated. Duplicate samples will be taken at each time point (approximately 14) for three conditions and two cell types. (Flight 84 samples. Total 168)

D. Measurements/Sample Handling:

Preflight: Cell preparations.

In-flight: Mix cells and mediators initially, remove samples at selected times and fix.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Protein kinase C evaluation, characterization of microtubule and actin cytoskeleton, gene expression by *in situ* hybridization.

F. Experiment Controls:

In-flight: 1-g and the microgravity centrifuge at 1-g.

Ground: Parallel conduct of the ground control during flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

A 37°C tissue culture incubator, laminar flow hood, a 20°C (or 4°C) refrigerator, and a centrifuge to provide 1-g on orbit (-187°C freezer is desirable for alternative sample storage).

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, work area, temperature controlled laboratory centrifuge, refrigeration (4°C), snap/quick freezer, -196°C freezer.

Experiment Identification Code: CB-b C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. Ernest J. Peck & Steve Carper

Science and Mathematics, UNLV
4505 South Maryland Pkwy
Las Vegas, NV 89154-4001

Critical Question(s) Addressed:

CB-1, CB-3 (See p. A-3)

3. Experiment Title

Assessment of Cellular Stress in Microgravity or Hypergravity

4. Purpose/Hypothesis

Cells exposed to microgravity or hypergravity may differ from controls because they have been stressed.

5. Scientific Rationale/Rationale for Microgravity

Stresses other than heat (eg, hypoxia, pH changes, heavy metals) induce the expression of heat shock proteins. This heat shock response is shown by all cell types from bacteria to man. It is probable that a change in gravity induces the expression of heat shock proteins. The presence of heat shock proteins (hsp) in any biological sample derived from space experimentation could be a very useful marker for the general stress state of the cell.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cell cultures will be maintained under 0-g and 1-g conditions. At intervals replicate samples will be taken from each culture and frozen (-196°C). Samples will be assayed by SDS-PAGE or two-dimensional gel electrophoresis and Western analysis postflight. Induction of specific heat shock proteins can be detected by quantitative densitometry postflight, or in more complex experiments, by labeling cells with radioactive amino acid precursors in-flight.

C. Number and Type of Specimen:

Two or more cell types (lymphocyte-derived and an endothelial cell line) will be used. Approximately 8 duplicate samples would taken for each condition (4) for a total of 64 samples.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks.

In-flight: Set-up cell cultures, remove samples at 2-4 day intervals from each culture and freeze at -196°C.

Postflight: Electrophoretic and Western analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Extract proteins from frozen samples, separate by two-dimensional or SDS-PAGE electrophoresis and detect heat shock proteins by Western blotting. Densitometry comparison of hsp expression under different experimental conditions.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, sterile sampling/set up environment (laminar flow hood), -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, work area, radioisotope handling equipment, temperature controlled laboratory centrifuge, snap/quick freezer, -196°C freezer, -70°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-c C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. G. William Fortner

Krug Life Sciences
1290 Hercules, Suite 120
Houston, TX 77058

Critical Question(s) Addressed:

CB-1, CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on Lymphocyte Differentiation

4. Purpose/Hypothesis

The effects of microgravity on cell division, cytokine production and effector function depend on the differentiation state of the cell at the time of exposure.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have shown that microgravity can reduce the rate of lymphocyte proliferation and block lymphocyte function. A study examining the effects of microgravity on the various steps of the lymphocyte differentiation pathway will provide valuable knowledge of cellular function in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured and stimulated in flight in both 0-g and 1-g conditions. Mitogen will be added and replicate cell samples taken daily from each culture and frozen (-196°C). Cytokine production and messenger RNA transcription will be assessed postflight.

C. Number and Type of Specimen:

Lymphocyte and lymphocyte-derived cell lines that constitutively and inducibly produce cytokines will be used. Approximately 12 duplicate samples from each experimental condition (4) would be collected (96 samples).

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks and mitogens.

In-flight: Set up cell cultures, remove daily samples and freeze.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Cytokine determination by ELISA and mRNA isolation and characterization by in situ-hybridization.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, laminar flow hood, microtiter plate which will allow mixing, -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, work area, temperature controlled laboratory centrifuge, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-d C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Terry C. Johnson

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3, DA-3 (See p. A-3, A-6)

3. Experiment Title

Cellular Differentiation Induced by A Proliferation Inhibitor

4. Purpose/Hypothesis

Several benchmark studies, already conducted in microgravity, suggest that in at least some cases cellular differentiation may be compromised. This has been emphasized by observations concerning the immune response and developmental defects in certain avian systems. The purpose of these experiments is to study the ability of a cell growth inhibitor to induce cellular differentiation in microgravity when mitotic arrest is mediated with certain human leukemic cells affected by this inhibitor.

5. Scientific Rationale/Rationale for Microgravity

There is a need to develop simple and straightforward model systems to study molecular events associated with cellular differentiation in microgravity. Since metazoan species largely depend on cell-cell communication and molecular networking to properly coordinate developmental programs among tissues, and each of these events is potentially impacted by the microgravity environment, limiting experiments to ground-based studies will certainly prove insufficient for extrapolation to the microgravity environment. The lack of information concerning the microgravity surely will prove to be a disabling feature of potential use of the Space Station and future space exploration.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 30-60 days

B. General Approach Description:

A nontoxic inhibitor of cell cycling will be used to maintain the cells in mitotic arrest and to induce the series of events that leads to cellular differentiation while they are stalled in G0/G1 of the cell cycle. This already has been demonstrated by ground-based studies, although the potential influence, either direct or indirect, of the microgravity environment remains unknown.

C. Number and Type of Specimen:

Human HL-60 leukemic cells are grown in suspension, and mouse neuroblastoma cells are grown in monolayer cultures throughout the experiment. Other cells of interest can be added to the protocol once these have been assessed on the Space Station.

D. Measurements/Sample Handling:

Preflight: Prepare cultures.

In-flight: Maintain cell cultures, add inhibitor and normal medium -without inhibitor- to matched sets of cell cultures of each cell line. Measure number of cells at various intervals; observe morphological changes; add reagents that serve to measure metabolic differentiation.

Postflight: Remove cell cultures for continued incubation at unit gravity.

E. Specific Sample Analysis:

In-flight: Cell enumeration, microscopy for morphological analysis, a series of biochemical analyses to measure metabolic markers of cellular differentiation.

Postflight: Cell culture.

F. Experiment Controls:

In-flight: Cultures propagated without the inhibitor, but with coordinate changes in culture medium will be used to compare the experimental samples.

Ground: Same as in-flight.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Incubator for both suspension and monolayer cultures; facilities to subculture cells; small centrifuge (2000 x g); inverted phase contrast microscope to observe cells grown in monolayer culture; fluorescence microscope; colorimeter and spectrophotometer; facilities to enumerate cell number per culture (electronic or microscopic); refrigerator; freezer (-20°C) and ultra-cold freezer (-80°C).

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, work area, temperature controlled laboratory centrifuge, compound microscope, data storage, spectrophotometer, refrigeration (4°C), -20°C freezer, -70°C freezer, fluid handling tools.

Experiment Identification Code: CB-e C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Robert J. Kinders

Head, Binding Agents Development
Molecular Diagnostics Research D907
Diagnostics Division, Abbott Laboratories
North Chicago, IL 60064

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on Expression of Adhesion Mediating Tumor-Specific Glycoproteins

4. Purpose/Hypothesis

Cellular synthesis, cell surface expression, and secretion of adhesion mediating molecules could be altered quantitatively or qualitatively under microgravity conditions.

5. Scientific Rationale/Rationale for Microgravity

Cell-cell adhesion, and secretion of proteins are critical functions of vertebrate cells. Since glycosylation is a key element of adhesion properties and secretion of macromolecules, and since O-linked glycosylation events are epigenetic and quite variable while N-linked glycosylation events are not, an assessment of expression of these events at the molecular level is warranted.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cell line LS174T (human colon ca) will be plated and cultured in both 0-g and 1-g conditions in 24-well plates. Each day, culture supernatants will be removed and vialled (separately for each well), and both the cells and supernatants will be frozen (-80°C or colder). mRNA, cell surface glycoproteins CEA and TAG-72, and secreted CEA and TAG-72 will be assessed by MAb and lectin binding postflight. A cell growth curve will be determined postflight.

C. Number and Type of Specimen:

Two 24-well plates and 48 vials of cell culture supernatants per day for fourteen days after the two cultures are seeded. Only one cell line will be necessary.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks and tissue culture media.

In-flight: Plate out cell culture; collect and freeze samples daily.

Postflight: (Maintain frozen samples).

E. Specific Sample Analysis:

In-flight: View cells under phase contrast microscope to monitor cell adherence and estimate cell density (by field counts if possible).

Postflight: Cell counts; mRNA collection and analysis; cell membrane glycoprotein analysis by MAb and Lectin binding; cell culture supernatant analysis by immunoassay. Electrophoresis devices, plate ELISA reader, ultracentrifuge, and slide centrifuge required.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Parallel experiment with cells from identical passage, and extensive historical data.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ incubator, pipettors (positive displacement), laminar-flow hood, phase-contrast microscope, mechanical cell count recorder, ultra-cold freezer, 1-g CO₂ incubator.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, (temperature controlled chamber), work area, fluid handling tools, compound microscope, data storage, -70°C freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-f C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Beth A. Montelone

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Stress Protein Induction in Microgravity

4. Purpose/Hypothesis

The stress protein (heat shock) response is ubiquitous among organisms and is induced by a large number of external agents. We will examine the functioning of this important protective pathway in microgravity using the model eukaryote *Saccharomyces cerevisiae*.

5. Scientific Rationale/Rationale for Microgravity

Cells perceive alterations in their surroundings by complex regulatory mechanisms involving cytoskeletal elements. These may be altered in microgravity, resulting in defects in the cell's ability to detect and respond to potentially dangerous situations. Alternatively, cells may respond to microgravity by constitutive expression of stress proteins. Potential consequences are unknown.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Yeast cells will be grown in culture at low temperature. They will be shifted to high temperature or known stress protein inducers will be added to the culture. After various time, cells will be pulsed-labeled with ³⁵S-methionine chased with cold methionine, collected and stored at -70°C for postflight analysis of protein.

C. Number and Type of Specimen:

Duplicate cultures of laboratory yeast strains will be used in experiments involving heat shock and induction by ethanol and heavy metals; one set of controls will be included (total of 8 cultures; 4 samples prepared from each culture for a total of 32).

D. Measurements/Sample Handling:

Preflight: Prepare strains.

In-flight: Start cell cultures; add inducer; remove samples at intervals, add label; incubate; chase; harvest cells by centrifugation. Freeze cell pellets.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Preparation of protein extracts and SDS-PAGE analysis.

F. Experiment Controls:

In-flight: None.

Ground: Parallel cultures to flight samples.

7. Hardware Requirements

A. Minimum on Orbit Requirements

23 and 37°C refrigerated incubators with agitation (not CO₂); sterile sampling environment (hood); -70°C freezer; radioactive isotope handling equipment; microcentrifuge.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, -70°C freezer, temperature controlled laboratory centrifuge, work area, fluid handling tools, radioisotope handling equipment, refrigeration (4°C).

Experiment Identification Code: CB-g C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. George L. Marchin

Division of Biology
Kansas State University
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on Gene Expression and Phage Production by Bacteriophage T4

4. Purpose/Hypothesis

The sequence of T4 gene expression and burst size will be affected by phage development in microgravity.

5. Scientific Rationale/Rationale for Microgravity

T4 phage and its *vs* gene mutants exhibit a precise and predictable, and similar sequence of gene expressions and burst sizes at 1-g. Many of the events in T4 gene regulation: DNA injection, effector diffusion, protein-protein interaction might alter as a result of microgravity. Therefore, determination of appearance of an immediate early, delayed early, and quasi-late enzyme as well as burst sizes in a mutant and wild-type pair under conditions of microgravity might differ.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Escherichia coli B will be cultured in-flight in microgravity condition. Two samples will be infected, one with wild-type T4 and one with a *vs* gene mutant. Successive samples will be taken, centrifuged, and frozen for enzyme analysis postflight. Several samples will be taken, CHCl₃ added and returned for postflight burst size analysis.

C. Number and Type of Specimen:

Each infected culture will yield 10 frozen samples for enzyme assay and one sample for burst size analysis. Total of 22 samples.

D. Measurements/Sample Handling:

Preflight: Phage lysate bacterial culture.

In-flight: Grow bacteria, add phage, take samples.

Postflight: Measure enzymes, determine burst.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay valyl-tRNA synthetase, dCMP deaminase, dTMP kinase activity. Determine burst.

F. Experiment Controls:

In-flight: Plate surviving bacteria.

Ground: Data are known. Could repeat.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Incubator, centrifuge, freezer, spectrophotometer (vis).

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, (temperature controlled chamber), data storage, temperature controlled laboratory centrifuge, -70°C freezer, spectrophotometer (vis) , work area, fluid handling tools, refrigeration (4°C).

Experiment Identification Code: CB-h C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Joseph S. Murray

Division of Biology
Kansas State University
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Activation of T cell Lymphokine Genes with Monoclonal Antibodies to the T cell Receptor

4. Purpose/Hypothesis

Activation of T lymphocytes through the alpha/beta component of the T cell receptor generates distinct patterns or levels of lymphokine gene expression in 0-g versus 1-g conditions.

5. Scientific Rationale/Rationale for Microgravity

T lymphocyte activation (as measured by thymidine incorporation) by the mitogen Concanavalin-A has been reported to be impaired during space flight. Whether activation of T cells by the physiological ligand of antigenic peptide plus MHC is similarly impaired is not known. Furthermore it is not known whether microgravity impairs or augments the activation of lymphokines which control humoral and cell-mediated immunity. As a start we will determine if activation by a monoclonal antibody directed to the portion of the T cell receptor which interacts with its physiological ligand leads to different levels or patterns of lymphokines expressed by T cells in space.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cloned T cell lines and short-term T cell cultures will be incubated with or without the monoclonal antibody at 0-g and 1-g. Duplicate samples (10^6 cells) will be harvested daily (approximately 12, 24, 48, 72, 96 h) and frozen (-196°C). Messenger RNA will be analyzed by in situ hybridization with sense- and antisense-strand probes to the lymphokines interleukin -2, interleukin-4 and interferon-gamma postflight.

C. Number and Type of Specimen:

Cloned and short-term murine T lymphocytes will be utilized and approximately 15 duplicate samples will be measured at 0-g and 1-g (60 total samples).

D. Measurements/Sample Handling:

Preflight: Preparation of T cells - adjustment of viability and cell numbers.

In-flight: Mixing monoclonal antibody, mitogen, and media control solutions with T cell suspensions. Addition of freezing media and the freezing of samples.

Postflight: Removing frozen cells and fixation.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Hybridization with probes for lymphokine mRNA and determination of mRNA steady-state levels by image analysis.

F. Experiment Controls:

In-flight: 1-g controls for all experimental conditions.

Ground: Duplication of in-flight 1-g experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ incubator at 0 g and 1 g, -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, snap/quick freezer, -196°C freezer, work area, refrigeration (4°C).

Experiment Identification Code: CB-i C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): David M. Klaus

BioServe Space Technologies
University of Colorado Campus Box 429
Boulder, CO 80309

Critical Question(s) Addressed:

CB-3, CB-8 (See p. A-3)

3. Experiment Title

Microgravity Effects on *E. Coli* Growth and Development

4. Purpose/Hypothesis

The growth and development of *E. coli* is gravity sensitive.

5. Scientific Rationale/Rationale for Microgravity

Changes in cell genetics, population growth rate, immunological properties, morphology and viability have been observed in previous flight experiments using *E. coli*. These studies, however, have formed inconclusive and often contradictory results. A systematic, long term approach is necessary to develop a statistically significant database defining the behavior of bacteria in the space environment. *E. coli* provides a useful, well documented model for these studies. This knowledge is important for the development of a Closed Ecological Life Support System (CELSS) and in understanding the potential alteration of bacterial vectored diseases on extended duration space habitation.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 (30 days)

B. General Approach Description:

Cells will periodically be inoculated into fresh growth medium to provide multiple generations of *E. coli* grown in space under microgravity and on a 1-g centrifuge for control. An autonomous apparatus will be used to perform fluid transfer and data monitoring and storage. Real time data collection will include optical density measurements to assess population growth. Samples will be taken at specified intervals and fixed or frozen for return. Post flight analysis will include a variety of assays for protein content, subsequent growth rates and morphology.

C. Number and Type of Specimen:

E. coli. The cells will be cultured in approximately 5 ml volume containers. A 1 ml sample will be drawn daily from duplicate experiments in microgravity and 1-g centrifuged samples. Fresh growth medium will be inoculated at 3 day intervals with the remaining saturated sample volume frozen. A total of sixty 1 ml fixed samples and ten 2 ml frozen, viable samples will be returned.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks and growth medium.

In-flight: Periodic sampling and inoculation, optical density measurement, freeze and fix samples.

Postflight: Maintain temperature control.

E. Specific Sample Analysis:

In-flight: Optical density measurement.

Postflight: Electrophoresis, enzyme assays, morphology, subsequent growth

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Parallel of flight experiments, clinostat and hyper-g experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

PI provided autonomous hardware, approximately 1.5 cu. ft. 500 W power, cooling interface for 0°C and 20-37°C.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, work area, data storage, spectrophotometer, -70°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-j

C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. R. William Currie

Department of Anatomy & Neurobiology
Dalhousie University
Halifax, Nova Scotia
B3H 4H7 Canada

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Effect of Microgravity on Expression of Heat Shock and Immediate Early Genes

4. Purpose/Hypothesis

To determine whether microgravity induces expression of immediate early genes or heat shock genes in cells and whether prior induction of the heat shock response can protect cells from the stresses of microgravity.

5. Scientific Rationale/Rationale for Microgravity

Our previous studies have shown that many physiological relevant stresses can invoke a heat shock response. In addition our studies demonstrate that prior induction of the heat shock response can significantly protect cells and improve recovery of myocardium from ischemic injury. Studies examining the effect of microgravity on gene expression and whether these alterations in gene expression can be attenuated by prior induction of the stress response will provide valuable information on cell metabolism and may lead to novel mechanisms for controlling cellular responses to microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured in flight in both 0-g and 1-g conditions. Replicate cell samples will be taken daily from each of the cultures and will be frozen (-185°C). mRNA transcription and protein translation will be assessed post-flight.

C. Number and Type of Specimen:

Cardiac myocyte cultures will be used. Duplicate samples (12) of each experimental condition (4) will be required (total of 96 samples).

D. Measurements/Sample Handling:

Preflight: Prepare cell cultures.

In-flight: Set up cell cultures, remove daily samples from each culture and freeze.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Northern and *in situ* hybridization analysis of RNA. Western and immunohistochemical analysis of proteins.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight cell cultures in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Cell incubator with humidity and CO₂ /O₂ control, 1-g cell incubator with humidity and CO₂ /O₂ control, laminar flow hood, inverted microscope, freezer (-185°C).

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, work area, fluid handling tools, compound microscope, temperature controlled laboratory centrifuge, data storage, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-k C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Joseph F. Albright

2. Author(s): Dr. Bruce A. Fuchs

Box 613 MCV Station
Department of Pharmacology & Toxicology
Medical College of Virginia/VCU
Richmond, Virginia 23298-0613

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Microgravity-induced Alterations in Agonist Induced Receptor Sequestration

4. Purpose/Hypothesis

To determine if microgravity is able to interfere with the normal process of agonist induced sequestration of a cell surface β -adrenergic receptors.

5. Scientific Rationale/Rationale for Microgravity

Previous experiments have suggested that a number of cytoskeletal elements are affected by microgravity. Agonist induced sequestration of cell surface receptors is partially regulated by the level of agonist in the environment. If this regulatory mechanism is compromised the responsiveness of the cells to drugs may well be altered.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells which have been cultured in-flight in both 0-g and 1-g conditions will be briefly exposed to agonist for short periods of time (~20 minutes). Following this exposure, the cells will be cooled to ice temperatures, incubated with various concentrations of radioligand specific to the receptor in question and then vacuum filtered onto glass fiber paper. Radioactivity on the glass fiber papers will be counted post-flight. Use of appropriate controls will allow for quantification of cell surface versus internalized β -adrenergic receptors.

C. Number and Type of Specimen:

A lymphocyte line known to express β -adrenergic receptors will be used. Ideally, 384 samples would be utilized to determine cell surface and total receptor concentration before and after agonist. This means that a total of 768 samples would have to be filtered to account for both 0-g and 1-g conditions. This might be easily accomplished using a cell harvester designed to filter from 24-96 samples at once.

D. Measurements/Sample Handling:

Preflight: Prepare cell stock and radioligand dilutions.

In-flight: Set up cultures. Incubate cells with agonist for 20 minutes at 37°C; incubate cells with radioligand for 60 minutes at 0°C. Filter samples onto glass fiber filter paper.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Count radioactivity on glass fiber filters. Perform Scatchard analysis to determine cell surface and total receptor density.

F. Experiment Controls:

In-flight: 1-g control.

Ground: 1-g cultures set up and treated in parallel with in-flight experiment.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, laminar flow hood for sterile cell manipulations, vacuum operated cell harvester.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, radioisotope handling equipment, data storage, work area, fluid handling tools, -70°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-1 C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. James E. Urban

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on the Swelling of Nitrogen-fixing Bacteroids in *Rhizobium* sp.

4. Purpose/Hypothesis

Microgravity will enhance the swelling of *Rhizobium* cells after their stimulation by bacteroid-inducing molecules.

5. Scientific Rationale/Rationale for Microgravity

Ground-based experiments characterizing bacteroid formation suggest that derepression of nitrogenase and other bacteroid enzymes is linked to the induction and amount of cell swelling and that swelling and consequent depression may be enhanced in the absence of gravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2, 90 days

B. General Approach Description:

Cells will be cultured and assayed in 0-g and at 1-g conditions. Samples will be exposed to inducer on day one to allow automatic collection and formaldehyde fixation of replicate samples at 15 day intervals. The degree of swelling and relative amount(s) of derepressed enzymes present will be determined postflight.

C. Number and Type of Specimen:

Rhizobium bacterial cells and the inducer succinic acid will be used. Approximately 3 replicate samples will be collected at each interval ($3 \times 7 = 21$ total samples).

D. Measurements/Sample Handling:

Preflight: Prepare cells and inducer for mixing, prepare formaldehyde fixative.

In-flight: Mix cells and inducer on day one, sample immediately and at 15 day intervals thereafter.

Postflight: Assay swelling microscopically and enzymes immunologically.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Collect cells by centrifugation, quantitate swelling and enzymes.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Ground (1-g) controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

A 30°C shaking incubator in a 1-atm environment; capabilities for sterile cell handling, automated sampling and formaldehyde addition.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, refrigeration (4°C).

Experiment Identification Code: CB-m C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Larry Takemoto & Dr. Beth Montelone

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Transport of Recombinant Protein in *E. Coli*

4. Purpose/Hypothesis

Microgravity may influence membrane-associated processes. One of these important processes involves protein transport across the membrane. These experiments will determine if microgravity alters the transmembrane passage of a recombinant protein.

5. Scientific Rationale/Rationale for Microgravity

Previous experiments have suggested that membrane-associated processes in the cell may be influenced by microgravity. Transport of proteins across the membrane is a necessary step in the assembly and secretion of many biologically important proteins. Using a recombinant DNA containing a signal sequence, these experiments will test whether microgravity will affect transport across the plasma membrane of *E. coli*.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

E. coli cells infected with a plasmid containing the sequence for the protein alpha crystallin plus the OmpA signal will be grown at 0-g and 1-g. Recombinant protein expression will be induced with IPTG, then incubated for 0-4 hrs, followed by freezing at -20°C. The rate of alpha crystallin transport across the plasma membrane into the periplasmic space will be assessed postflight.

C. Number and Type of Specimen:

E. coli infected with a plasmid will be used in all experiments.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks.

In-flight: Induce protein expression with IPTG, incubate at 37°C for 0-4 hrs, freeze samples for postflight analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Quantify amount of expressed alpha crystallin in cytosolic and periplasmic spaces.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Cells will be treated in an identical manner to those in microgravity.

7. Hardware Requirements

A. Minimum on Orbit Requirements

37°C incubator, -20°C freezer, bioprocessing modules.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, -20°C freezer, work area, refrigeration (4°C).

Experiment Identification Code: CB-n C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. Mary Lee S. Ledbetter

Department of Biology
College of the Holy Cross
One College Street, P. O. B
Worcester, MA 01610

Critical Question(s) Addressed:

CA-6, CB-3 (See p. A-3)

3. Experiment Title

Microgravity Effects on Gap-junction Mediated Cell-Cell Communication

4. Purpose/Hypothesis

Formation of communicating junctions among cultured animal cells and their effectiveness once formed is altered by microgravity.

5. Scientific Rationale/Rationale for Microgravity

Cells in culture, like cells in tissues, are able to form gap junctions, specialized structures that join the membranes of two cells and permit small molecules to diffuse readily between their cytoplasm. These junctions have been implicated in coordination and regulation of cell growth, differentiation and metabolism. I know of no information that relates to their formation or function in microgravity, but effects might be anticipated due to mechanical influences on cell-to-cell contact and on membrane responses.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured in flight in both 0-g and 1-g conditions. Periodically samples will be frozen for freeze-fracture microscopy, fixed for thin-section microscopy, and frozen for immunocytochemical analysis of distribution of connexin protein. Subgroups of cells will be radiolabeled with ³H-Uridine, plated onto unlabeled cells, and after an interval to permit exchange of nucleotides, fixed for light microscope autoradiography. Microscopic preparation and observation will be accomplished postflight.

C. Number and Type of Specimen:

Two cell lines will be used, a fibroblast line, such as 3T3, and an epithelioid line, such as MDCK. Both are attachment-dependent for growth. Daily sampling in triplicate over the second through 15th days of flight would generate 84 samples for each type of observation. Leighton tube cultures are the preferred mode for the samples; stock cultures can be grown in tissue-culture flasks.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks, distribute coverslips and growth medium to Leighton tubes, prepare radiolabeled growth medium and fixatives.

In-flight: Maintain cell stocks (change medium every three days, subculture every six days), set up test cultures, prepare labeled cells and plate them onto the test cultures, sample regularly as above. Fix/freeze samples.

Postflight: Maintain samples for analysis.

E. Specific Sample Analysis:

In-flight: Assess health of cultures; monitor numbers of cells plated.

Postflight: Immunohistochemical staining for location and amount of connexin protein, autoradiographic analysis of exchange of nucleotides, freeze-fracture and thin-section electron microscopy for number and area of gap junctions.
Darkroom, anti-connexin antibodies, electron microscopy facility, and high quality fluorescent-phase contrast microscopes with photographic capability and image analysis required.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight 1-g experiments.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Cell incubator, either CO₂ controlled or with gas available to equilibrate sealed cultures, both at 0 g and 1 g. Inverted phase contrast microscope for observation. Containment areas for isotopically labeled materials. Storage for fixed specimens (cells on coverslips) at room temperature and for frozen specimens at -196°C.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, radioisotope handling equipment, compound microscope (with fluorescence sensitivity), data storage, fluid handling tools, work area, snap/quick freezer, -196°C freezer, -70°C freezer, refrigeration (4°C), ambient storage.

Experiment Identification Code: CB-o C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Rose Grymes

NASA Ames Research Center
MS 239-11
Moffett Field, CA 94035

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Viral Replication and Packaging in Microgravity

4. Purpose/Hypothesis

The nature of viral replication and reproductive events may differ in microgravity from those observed at 1-g.

5. Scientific Rationale/Rationale for Microgravity

Viral reproduction occurs at two levels: 1) subversion of cellular replication machinery to generate multiple new copies of viral genetic information (DNA or RNA) and viral structural proteins, and 2) appropriate special assembly of these components into multiple 'daughter' virions. Alterations of cellular metabolism as a result of microgravity exposure may alter the production of viral components. Gravity requirements for correct spacing and assembly of viral proteins may result in defective (or accelerated) viral production.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Normal mammalian cells will be cultured in flight in both microgravity and 1-g conditions. Cells will be infected with a retrovirus (RNA genome) to which they are susceptible. Several points in the viral replication cycle will be examined--production of double stranded DNA intermediate, activity of in-put reverse transcriptase viral enzyme, pulse-chase labeling of viral RNA, identification of viral polypeptides and fusion proteins by immunoprecipitation and Western blotting, antibody staining of viral proteins assembled at the cell membrane, electron micrography of budding viral particles. The titer of virus produced, and the infectivity of these microgravity produced stocks will be assessed post-flight.

C. Number and Type of Specimen:

Microgravity and simultaneous on-board 1-g controls are required. All other operations can be accomplished by appropriate sampling of large cultures (bio-reactor) or by parallel treatment of smaller cultures.

D. Measurements/Sample Handling:

Preflight: Initiate cell cultures, load viral stock and fixatives/isolation treatments.

In-flight: Infect cells with virus, isolate/fix specimens according to schedule.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Isolate/restriction map double-stranded DNA intermediate, measure reverse transcriptase activity, quantify viral proteins (immunoprecipitation, Western blotting), antibodies to viral proteins used to stain infected cells, electron micrography, assay the titer of new virus stocks.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, sampling equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, refrigeration (4°C).

Experiment Identification Code: CB-p C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Christopher C. Field

Southwest Missouri University
Department of Biomedical Science
901 S. National Avenue
Springfield, MO. 65804-0094

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Differential Oncogene Expression Induced by Microgravity

4. Purpose/Hypothesis

As major regulators of cellular activities, oncogenes will be altered in their expression by microgravity and will initiate a changed cascade of cellular responses.

5. Scientific Rationale/Rationale for Microgravity

Oncogenic proteins are diversified effectors of cellular activities and trans activating substances that ultimately lead to transformation of the cell. Because of their diversified effects on the cell, one or several of the oncogenes may be expressed in microgravity and initiate the cascade of events leading to muscle atrophy, altered calcification of bone or other cellular changes observed in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 21 days

B. General Approach Description:

NIH 3T3 cells and several NIH 3T3 cell lines receiving different, single oncogene promoter sequences attached to a reporter system of chloramphenicol acetyltransferase (CAT) will be grown in 0-g and 1-g. Oncogenes will be selected from the major functional classes. Samples will be collected at 6 hour intervals for the first 48 hours of flight and every third day for the remainder of the flight. Samples will be frozen at -196°C for analysis postflight.

C. Number and Type of Specimen:

Four samples of each cell type will be analyzed: natural 3T3, and 3T3 receiving one of the following oncogene reporter systems PDGF, EGF, erbB, kit, gre, abl, ras, raf, jun, fos, and natural jun-fos for heterodimer formation, and myc. As 42 samples may be taxing to sample, selected oncogenes will be used initially and the remainder flown later.

D. Measurements/Sample Handling:

Preflight: Initiate growth of normal and transfected cells in Kearney Growth Chambers.

In-flight: Collect samples at specified times and freeze.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analyze samples from normal 3T3 cells for oncogene products by PAGE-Western blot detection using monoclonal antibodies for oncogene products and assay transfected cells by the standard CAT detection system.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Kearney Orbital Growth Chambers, low volume size, with sterile sampling and liquid nitrogen freezing capacity.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, work area, temperature controlled laboratory centrifuge, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-q C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. Jeffrey R. Hazel & Dr. David G. Capco

Molecular and Cellular Biology Program
Department of Zoology
Arizona State University
Tempe, AZ 85287-1501

Critical Question(s) Addressed:

CB-3, CB-6, CB-7 (See p. A-3)

3. Experiment Title

Cellular Adaptations to Microgravity in Signal Transduction Mechanisms

4. Purpose/Hypothesis

Two hypotheses will be tested: 1) microgravity will perturb the β -adrenergic signal transduction pathway; and 2), cells exposed to microgravity for an extended period will compensate for the effects of microgravity on the β -adrenergic transduction pathway.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have suggested that signal transduction mechanisms (e.g. mitogenic stimulation of lymphocytes) may be perturbed by microgravity. The present experiments will determine i) the extent to which the β -adrenergic signal transduction mechanism is perturbed by microgravity in mammalian cell lines whose growth is both anchorage-dependent and independent by quantifying the amount of cyclic AMP produced in response to a defined dose of β -adrenergic agonist, and ii) the extent to which the capacity for cAMP production recovers from (compensates for) extended exposure to microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured in-flight under both 0-g and 1-g conditions. Cells in culture will be exposed to β -adrenergic agonist (isoproterenol, 10 μ M) for 1 minute, at which time they will be harvested and frozen (-196°C) for post-flight analysis of cellular cAMP levels. Cellular response to receptor agonist will be determined in triplicate cell samples obtained on alternate days for the duration of a 16-day flight.

C. Number and Type of Specimen:

3T3-L1 and S49 murine lymphoma cells will be used as the anchorage-dependent and independent cell lines, respectively. Triplicate samples from each of the two cultures will be harvested at 2-day intervals for a total of 48 samples.

D. Measurements/Sample Handling:

Preflight: Prepare cell cultures.

In-flight: Maintain cultures, incubate cultures with receptor agonist, collect and freeze samples at 48-hr intervals for the duration of the flight.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of cAMP levels in collected samples using RIA.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: 1-g controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ incubator, 1-g cell incubator, rapid biochemical freezing (-196°C) capability.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, snap/quick freezer, -196°C freezer, work area, refrigeration (4°C).

Experiment Identification Code: CB-r C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Valerie E. Martindale

Clinical Investigation Directorate
Wilford Hall Medical Center
Lackland AFB
San Antonio, TX 78236-5300

Critical Question(s) Addressed:

CB-3 (See p. A-3)

3. Experiment Title

Investigation of Stress-Response Threshold in Microgravity

4. Purpose/Hypothesis

Many different agents and conditions induce a cellular stress response, characterized by production of some subset of the heat shock proteins. Each organism has its own, reproducible threshold for induction of the stress response. Hypothesis: this threshold is altered in the microgravity environment.

5. Scientific Rationale/Rationale for Microgravity

There are a variety of alterations in cell function in microgravity, which may be expected to be stressful to the cell. These alterations are expected to be subthreshold in most cell types, i.e. they will not of themselves induce a stress response. However, if they are stressful to the cell, they will lower the cell's threshold of response to other stressors. This effect may be uncovered by applying stressors, such as heat, at levels which would normally be subthreshold, to produce a stress response. The cytoskeleton has been identified as a likely focus for the effects of microgravity, and therefore the observation that cytoskeletal elements are disrupted by heat shock makes heat a good choice of added stressor, if additive effects are to be demonstrated.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured in-flight in both 0-g and 1-g conditions in multiple, independent culture chambers. At each time point to be investigated, one chamber from each will be heated to a temperature below normal heat shock threshold. Exact temperatures will depend on the cell type (see below). After 30 minutes, replicate cell samples from each of the four heated chambers will be harvested and frozen (-196°C). Messenger RNA for heat shock proteins, and cellular protein profiles, will be assessed postflight. Time points for investigation will be one hour after orbital insertion, and orbital days 1, 2, 4, and 8.

C. Number and Type of Specimen:

A stable, well characterized, immortalized cell line will be used. A *Drosophila melanogaster* cell line is a good choice. There are also well characterized mammalian cell lines available. A minimum of twenty small culture chambers with independent temperature control is needed at both 0-g and 1-g. Ideally, three times that number would be used to provide triplicate independent samples (total 60 samples).

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks.

In-flight: Set up cell cultures, apply heat stress (may be automated), remove samples and freeze at indicated times.

Postflight: Divide each sample in two, one for mRNA and one for protein analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Quantification of mRNA for HSP 70 and HSP 90, 2-dimensional protein electrophoresis and quantitative densitometry to compare protein profiles.

F. Experiment Controls:

In-flight: Positive heat shock control, 1-g control.

Ground: Controls will parallel 1-g in-flight control.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

CO₂ cell incubator with multiple (minimum 20) independently controlled, programmable chambers at both 0 g and 1 g; sterile sampling capability; -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, work area, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-s C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. George P. Kearney

Department Biosciences
Walter Reed Army Institute of Research
Washington, D. C. 20307-5100

Critical Question(s) Addressed:

CB-3, DB-3 (See p. A-3, A-6)

3. Experiment Title

Microgravity Effects on Protein Isoform Expression in Muscle

4. Purpose/Hypothesis

Artificial gravity applied in the form of centripetal acceleration will effect muscle changes in space flown tissue.

5. Scientific Rationale/Rationale for Microgravity

Alterations in protein composition and metabolic characteristics are known to occur in muscles of space flown animals. By following production of mRNA for the different myosin heavy and light chains and conducting inventories of deployed isotypes it is possible to chart the transition. These data will be used to pursue initial signal and control point determination.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 or Phase 3 (14-28 days)

B. General Approach Description:

Primary cultures and differentiated L-8 cells will be adapted to microcarriers both alone and in co-culture with neuroblast materials. The materials will be divided and subjected to ambient microgravity or pseudogravity. Aliquots will be drawn and fixed or frozen at -196°C.

C. Number and Type of Specimen:

Three replicate cultures for the two experimental groups.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Collect and fix or freeze samples.

Postflight: Maintain samples for analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Western and northern blotting of extracts. In situ hybridization of materials. Equipment for blotting and hybridization, electrophoresis set-up, and thermocycler for PCR amplification of transcripts required.

F. Experiment Controls:

In-flight: Centrifuge controls.

Ground: Ground-based controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ incubator, -196°C freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, snap/quick freezer, -196°C freezer, work area, refrigeration (4°C).

Experiment Identification Code: CB-t C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Joseph F. Albright

2. Author(s): Dr. James P. Hughes & Dr. David A. Prentice

Department of Life Sciences
Indiana State University
Terre Haute, IN 47809

Critical Question(s) Addressed:

CB-3, CB-4, DA-3 (See p. A-3, A-6)

3. Experiment Title

Effects of Microgravity on Neuronal Differentiation

4. Purpose/Hypothesis

Microgravity-induced changes in neurite extension will alter the intracellular distribution of the cytoskeletal proteins p11 and annexin II, and decrease phosphorylation of stathmin, a process closely associated with neuronal differentiation.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have shown that microgravity alters cytoskeletal organization and phosphorylation. These elements are critically important in neuronal differentiation, a process that is characterized by re-organization of the cytoskeleton that leads to formation of growth cones and the extension of neurites. This re-organization is orchestrated by a number of phosphorylation/de-phosphorylation reactions in which stathmin appears to play a critical role as an intracellular relay.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

PC-12 cells will be cultured in-flight under 0-g conditions. Replicate samples will be taken daily for analysis of p11/annexin II/stathmin. These samples will be frozen. Cells will also be fixed *in situ* for analysis of protein distribution by immunocytochemistry. All samples will be analyzed post-flight.

C. Number and Type of Specimen:

PC-12 cells, a pheochromocytoma cell line widely used as a model for sympathetic neuron differentiation, will be used. Six replicate samples from each of two conditions ($\pm$ Nerve Growth Factor) will be required (84 total samples).

D. Measurements/Sample Handling:

Preflight: Prepare cell cultures.

In-flight: Freeze/fix daily samples.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of p11/annexin II and the various forms of stathmin. Immunocytochemical analysis of same proteins.

F. Experiment Controls:

In-flight: $\pm$ Nerve Growth Factor.

Ground: Parallels of in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, sampling equipment, freezer (-190°C).

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, chemical fixation capability, data storage, snap/quick freezer, -196°C freezer, work area, temperature controlled laboratory centrifuge, refrigeration (4°C).

Experiment Identification Code: CB-u C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. G. June Marshall & Mary Kirchen

Los Angeles Orthopedic Hospital
2400 South Flower St.
Los Angeles, CA 90060

Critical Question(s) Addressed:

CB-5, DB-3 (See p. A-4, A-6)

3. Experiment Title

The Effects of Microgravity on Intracellular Calcium in Cultured Osteoblasts and Osteoclasts

4. Purpose/Hypothesis

Microgravity induced alterations of the cytosolic concentration of calcium will effect many cellular functions including motility, matrix formation and degradation, and mitotic rate. The purpose of this study is to investigate changes in cytosolic calcium of bone cells in order to understand the effects of microgravity on the metabolism of bone.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have shown that microgravity has a dramatic effect on calcium metabolism. Calcium is lost in both urine and feces and total calcium stores are not immediately replenished upon return to gravity. The exact cellular mechanisms by which calcium is lost has not been established. Since the depletion of calcium can interfere with most body functions, i.e. muscle contractility, cell mobility, cell adhesion and the formation of the bony matrix, an understanding of calcium loss at the cellular level in microgravity could open numerous pathways for understanding of calcium metabolism in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells will be cultured in flight at 0-g; comparable cultures will be maintained at 1-g on Earth and in a 1-g centrifuge in flight. Triplicate cell samples will be harvested daily from each of the cell cultures, placed in a cryoprotectant and frozen at -196°C. In addition, a fourth sample will be fixed for morphological studies by light and electron microscopy. Intracellular calcium and magnesium will be measured from frozen samples post-flight. Attempts will be made to re-establish thawed cultures post-flight so that proliferative rates and karyotypes can be studied.

C. Number and Type of Specimen:

Osteoblast and osteoclast cell lines. Approximately 16 quadruple samples from each experimental condition will be collected for a total of 128 samples.

D. Measurements/Sample Handling:

Preflight: Prepare cell cultures; cryogenic medium; fixatives.

In-flight: Maintain cell cultures, harvest daily samples, freeze and fix.

Postflight: Maintain samples prior to: calcium determinations, qualitative morphology studies.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Fluorescence spectrophotometry for calcium and magnesium, light and electron microscopy of cells, re-establishment and analysis of cells frozen in-flight.

F. Experiment Controls:

In-flight: Culture media alone, without cells.

Ground: Duplication of in-flight conditions on Earth to include microgravity and 1-g cells plus media and media without cells.

7. Hardware Requirements

A. Minimum on Orbit Requirements

37°C incubator with 7.2 to 7.4 pH control; capability for culture media exchange on scheduled time table; microcentrifuge with temperature and pH control; cell sampling capability; gluteraldehyde and formaldehyde fixation capabilities; liquid nitrogen freezing capability with frozen sample storage.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, temperature controlled laboratory centrifuge, chemical fixation capability, work area, data storage, snap/quick freezer, -196°C freezer, refrigeration (4°C).

Experiment Identification Code: CB-v C = cell; B = transduction/response

1. Discipline: Cell Biology

Reference SWG Member

Dr. William P. Wiesmann

2. Author(s): Dr. Edwin S. Miller, Jr. & Dr. Gerald Sonnenfeld

Department of Microbiology and Immunology,
School of Medicine
University of Louisville
Louisville, Kentucky 40292

Critical Question(s) Addressed:

CB-3, CB-4, CB-7 (See p. A-3)

3. Experiment Title

Influence of Space Flight on Immune Cell Function

4. Purpose/Hypothesis

These proposed experiments will evaluate the influence of space flight on immune cell function, which could provide information on how space flight alters physiological homeostasis and resistance to infection and cancer. The hypothesis is that space flight alters immunological parameters and resistance to infection.

5. Scientific Rationale/Rationale for Microgravity

Evidence from long- and short-term space flight studies has shown that space flight alters immune responses. The use of the Space Station will allow, for the first time, in-flight experimentation with immunologically important cells to determine the time of onset, duration, adaptation, and recovery from space flight-induced changes in immune responses.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3

B. General Approach Description:

Phase 1: Obtain blood samples from astronauts 24 h before flight, 24 h prior to landing and 24-48 h after recovery for ground-based analysis. Also fly tissue culture experiments for ground-based analysis.

Phase 2: Obtain blood samples from astronauts and blood, lymph node, spleen, and bone marrow samples from rodents before flight, 24 h prior to and 24 h after recovery for ground based analysis. Also fly tissue culture experiments for ground-based analysis.

Phase 3: Obtain blood samples from astronauts and blood, lymph node, spleen, and bone marrow samples from rodents before flight, and selected timepoints during flight, and after recovery for in-flight and ground-based analysis. In addition, non-human primates could be utilized.

C. Number and Type of Specimen:

Phase 1: Blood - sampled once before, once during, and once after flight. Tissue culture samples - human and murine macrophage cell lines. Phase 2: Astronaut blood and rodent tissue samples (blood, lymph node, spleen, and bone marrow) - sampled once before, once during, and once after flight. Tissue culture samples - human and murine macrophage cell lines. Phase 3: Astronaut and non-human primate blood sampled once before, at predetermined timepoints in-flight, and once after flight. Tissue culture samples - human and murine macrophage cell lines.

D. Measurements/Sample Handling:

Preflight: Analysis of macrophage function including cytokine production, cell surface marker expression, metabolic activities, response to cytokine therapy. Additional analysis on rodent samples for Phase 2 experiments and on non-human primates for Phase 3.

In-flight: Harvest samples (astronaut blood, rodent tissues, non-human primate blood) at specified timepoints (either 24 h before recovery for Phase 1 and 2, or at specified timepoints in flight for Phase 3).

Postflight: Same as preflight (for all flight phases).

*In each flight phase, some experimental analysis requires the use of live cells. After harvesting, samples must be maintained at either ambient temperature or at 4°C, depending on the tissue type and species source of tissues.

E. Specific Sample Analysis:

In-flight: Phase 3 only: Induction of cytokines with mitogens. PCR and bioassays for cytokine production and response to exogenous cytokines; PCR and flow cytometric analysis for cytokine receptor expression; enzyme assays and PCR for metabolic activities.

Postflight: For all flight phases: Same as in-flight for Phase 3. In addition, viable animals returned from flight can be infected with microbes or cancer cells to evaluate parameters of resistance.

F. Experiment Controls:

In-flight: When tissues or cell culture samples are harvested, aliquots of the sample will not be treated with an immunological challenge (i.e., mitogens).

Ground: Exact duplicate samples will be performed on the ground. If timing requires, delayed flight profile studies can be performed.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

UF: Ambient temperature storage. MTC: Ambient temperature and 4°C storage; tissue culture incubator; rodent holding facility. PMC: Same as in MTC plus, non-human primate holding facility, general purpose work station(s) for sampling and sacrifice of animals and for aseptic tissue culture experiments; general tissue culture lab facilities; flow cytometer; PCR apparatus; -70°C storage.

B. GBF Hardware Capabilities Required (on Orbit)

Phase 2: Cell culture apparatus, refrigeration (4°C), data storage, fluid handling tools, rodent weanling to adult habitat , work area, ambient storage, Phase 3 : Cell culture apparatus, refrigeration (4°C), data storage, fluid handling tools, rodent weanling to adult habitat , work area, -70°C freezer, ambient storage.

Experiment Identification Code: CC-a C = cell; C = other

1. Discipline: Cell Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. J.O. Kessler

Physics Department, Building 81
University of Arizona
Tucson, AZ 85721

Critical Question(s) Addressed:

CA-3, CC-3 (See p. A-3)

3. Experiment Title

Investigation of Foams, Applied to Microgravity Cell Culture Systems

4. Purpose/Hypothesis

To investigate suitability of foams as cell culture systems for space science and exploration.

(1) Foams whose liquid phases are constituted of cell culture media permit illuminated, well-aerated cell growth without constant shaking. (2) Drainage and liquid phase replenishment can be manipulated in microgravity so as to maintain nutrient concentration.

(3) Interaction of cells with the fluid boundary surfaces of the foam structure will modify cell division and cell morphology.

5. Scientific Rationale/Rationale for Microgravity

The establishment of foam systems for cell culture, and possibly also crystal synthesis, will provide a new method for manipulating growth parameters. Aeration, diffuse illumination, elimination of shear and elimination of the gravitational component of foam drainage are positive factors. Foam maintenance and liquid phase circulation require research. The clamping of cells in liquid films may generate unusual and instructive morphological variations in cells and other growing aggregates.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 (also Phase 2, Phase 3>90 days)

B. General Approach Description:

i) Study of foam "engineering": maintenance, fluid exchange methods (ground study, short space flights). Development of growth media foams.

ii) Test of cell growth in foamed media (various cell types, plant callus). Test of liquid exchange and illumination systems: preceded by ground experiments, "short" microgravity experiments.

iii) Long term tests of cell growth in foam culture, including growth rate, morphology, generation of new strains by selection pressure.

C. Number and Type of Specimen:

Algae (single cell, colonial, filamentary), at least six species. Other micro-organisms, bacteria, animal cells, plants. Choice depends on results of foam engineering studies.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Analysis of cell numbers and morphology, cell freezing, microscopy.

Postflight: Same as in flight.

E. Specific Sample Analysis:

In-flight: Growth rate, superficial shape, intracellular organization, motility (where applicable), intra/extra cellular changes in biosynthesis.

Postflight: Same.

F. Experiment Controls:

In-flight: Standard shaker culture.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Small handling systems for liquid, gases, foam generation. Aerosol retention system, illuminator and accelerator for small foam growth chambers, micro/macro scope, camera (35 mm), CCD/VCR. Special design chambers for foam experiments. Otherwise, standard hardware for analyzing morphology, biochemistry, cell concentration.

B. GBF Hardware Capabilities Required (on Orbit)

(Temperature controlled chamber), fluid handling tools, compound microscope, data storage, work area, spectrophotometer, refrigeration (4°C).

Experiment Identification Code: CC-b C = cell; C = other

1. Discipline: Cell Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Terry C. Johnson

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

CB-3, CB-4, CC-3 (See p. A-3)

3. Experiment Title

Transport of Cell Cultures in Mitotic Arrest

4. Purpose/Hypothesis

There is a vital need to maintain cell cultures in "suspended animation" while they are being transported to the Space Station by shuttle flights. Delays in launch, actual transportation, docking at the Space Station and/or securing the cultures in proper incubation chambers will require an ability to maintain unattended cell cultures, in suspended animation without scheduled feeding, for hours or days. A nontoxic cell surface inhibitor has been purified and characterized that holds cell in mitotic arrest for at least 48 to 55 hours without feeding, and may circumvent the inevitable dangerous overgrowth during this transportation period.

5. Scientific Rationale/Rationale for Microgravity

Although the cell surface inhibitor has been shown to function efficiently, with cells obtained from a wide variety of species, there presently is no evidence that its necessary interaction with cell surface receptors, and the ensuing disruption of early signal transduction that maintains the cells in mitotic arrest, will function in the microgravity environment. The use of the inhibitor, therefore, may be limited to prelaunch periods. In contrast, the efficient action of the inhibitor in the microgravity environment would lend itself to actual transport and for extended periods on the Space Station when the crew could 'activate' -on demand- the cells for experimental purposes.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Cells propagated in monolayer and suspension will be incubated with and without the cell cycle inhibitor. The number of cells per culture will indicate whether or not the inhibitor functions in arresting cell proliferation in the microgravity environment. Direct cell numbers will be assessed post-flight and compared to similar cultures treated in a similar manner in ground-based conditions. Further analysis of reversibility, DNA content and DNA synthesis will add information concerning the nontoxicity of the inhibitor in microgravity.

C. Number and Type of Specimen:

A variety of cell types in monolayer and suspension culture, fibroblasts and epitheliod, transformed and nontransformed, and from a wide variety of species from insect to avian to mammalian (all shown to be sensitive to the inhibitor in ground-based experiments).

D. Measurements/Sample Handling:

Preflight: Prepare cultures, with and without inhibitor.

In-flight: Maintenance of cultures under proper temperature, humidity and atmosphere.

Postflight: Removing cultures for immediate analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Cell enumeration, phase-contrast microscopy, DNA content analysis and reversibility of the mitotic arrest if the cultures remain inhibited. Requires: radioisotope facility to measure DNA synthesis after reversal of in-flight and ground-based cultures; FAX-Scan to measure DNA content per cell; and electronic cell counter to enumerate cell number per culture.

F. Experiment Controls:

In-flight: Comparable cultures maintained in identical medium and culture environment, but without the inhibitor.

Ground: Same as in-flight

7. Hardware Requirements

A. Minimum on Orbit Requirements

Facilities for maintaining cell cultures under proper conditions. Phase-contrast microscope to periodically monitor cultures.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, compound microscope, data storage, refrigerator (4°C).

Experiment Identification Code: DA-a D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

D.K. Holly Hayes

2. Author(s): Dr. Dora K. Hayes

U.S. Department of Agriculture
ARS - BA - LPSI - LIL
Beltsville, MD 20705-2350

Critical Question(s) Addressed:

DA-1 (See p. A-6)

3. Experiment Title

Effects of Microgravity on Diapause (Hibernation) in the Gypsy Moth

4. Purpose/Hypothesis

To determine whether the conditions of space flight accelerate insect development - specifically - maturation and eclosion of the embryonated, diapausing gypsy moth egg.

5. Scientific Rationale/Rationale for Microgravity

In nature, ninety days of chilling are required to terminate diapause. A previous flight study has shown that embryonated gypsy moth eggs (in diapause for 2 months) terminated their hibernating state early with only 10% of the chilling (10 days at -11°C) normally required for initiating development. In this proposed test, the eggs would not be chilled. Diapause termination may be a result of release of neuropeptide and other hormones from endocrine organs. This release may not be the result of repositioning of neurosecretory granules and disruption of the membranes of these subcellular organelles.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Gypsy moth egg masses will be incubated in 0-g and 1-g conditions in ambient temperatures and pressures in the GBF for the duration of the flight. Hatching or eclosion will be determined after termination of the space flight. (Differentiation among effects of microgravity, radiation and increased gravitational force of launch and reentry is not proposed.) A small sample of eggs will be examined by light and electron microscopy before and after the flight to determine whether differences in sub-cellular organelle staining result from the treatment. It would be useful to apply the techniques of molecular biology to determine whether space flight has altered expression of the genes affecting development. This should only be undertaken if suitable relevant background information is available.

C. Number and Type of Specimen:

100 gypsy moth egg masses, 75 - 400 eggs/egg mass, 68 egg masses from laboratory colony and 40 from wild population. Half to be used in 0-g and half in 1-g on GBF.

D. Measurements/Sample Handling:

Preflight: Place eggs in individual mesh bags and/or plastic petri dishes and load into approved flight container.

In-flight: Little or no crew interaction.

Postflight: Obtain 10 eggs from each egg mass and fix for electron microscopy. Place remaining egg masses in photoperiodic regimen of LD 14:10 at 12°C and observe hatch on a daily basis. Count shells and unhatched egg masses to determine the numbers of eggs actually in the test.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Evaluate emergence and light and electron microscopy data, especially in regard to neurosecretory cell activity or lack thereof compared to observed eclosion.

F. Experiment Controls:

In-flight: (as indicated above) 1-g controls.

Ground: 30 lab-reared egg masses and 20 field-collected egg masses.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Containers to hold 60 mm petri plates in temperature and atmosphere of the GBF.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage.

Experiment Identification Code: DA-b D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. John Morrill

Division of Natural Sciences
5700 N. Tamiami Trail
Sarasota, FL 34234-2197

Critical Question(s) Addressed:

DA-2, DB-2, DB-3, DG-3 (See p. A-6)

3. Experiment Title

Behavior of Migratory Cells and Cilia Formation During Development

4. Purpose/Hypothesis

It appears that microgravity effects cytoskeletal components of attachment dependent cells. Therefore, cells that migrate using exploration via filapodia may well be effected in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Some cytoskeletal components have been shown to be sensitive to pressure and may therefore be sensitive to various gravitational environments. Cilia and the leading edges of crawling cells are examples of sensitive cytoskeletal components whose cellular functions are easily observed. In vivo visualization of overall behavior as well as specific aspects of the cytoskeleton in crawling cells makes them excellent candidates for experimentation in microgravity. If these same cells were fixed, their cytoskeletal structures could be examined in detail and correlated to the behaviors observed while the cells were in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Both video microscopy and sample preservation will be used.

C. Number and Type of Specimen:

Sea Urchins and/or pond snails.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Video microscopy and sample preservation.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of cell migration from video images. Analysis of cilia formation.

F. Experiment Controls:

In-flight: None.

Ground: Embryos in one gravity.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Habitat chambers and video microscopy equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (fresh water / salt water), data storage, compound microscope, chemical fixation capability, ambient storage.

Experiment Identification Code: DA-c D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Bruce J. Crawford

Faculty of Medicine, Department of Anatomy
University of British Columbia
Vancouver, British Columbia
V6T 1Z3 CANADA

Critical Question(s) Addressed:

DA-2, DB-1, DG-1, DG-3 (See p. A-6)

3. Experiment Title

Microgravity Effects on Early Starfish Morphogenesis

4. Purpose/Hypothesis

To determine how microgravity effects cell/cell and cell/ECM interactions which are necessary for early development.

5. Scientific Rationale/Rationale for Microgravity

Studies of early development in microgravity should add to our knowledge of how gravity affects the cell/cell and cell/surface interactions which are necessary for normal development.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Embryos in early developmental stages will be allowed to undergo development at 0-g. Samples will be taken and fixed for light microscopy, scanning and transmission electron microscopy on days 1, 3, 5, 6, 7, 12, and 16. The effects of microgravity on early development will be assessed post flight.

C. Number and Type of Specimen:

Embryos from the starfish *Pisaster ochraceus* will be used. Two duplicate samples of 50-100 embryos each from each time point will be required (28 samples in all).

D. Measurements/Sample Handling:

Preflight: Obtain gametes, fertilize embryos and grow for approximately 1 day to ensure healthy cultures. Place in specimen chambers. (Rearing of embryos requires 12°C incubator, dissecting microscope, refrigerated sea water supplies and glassware.)

In-flight: Set chambers in incubator (1/2 on centrifuge at 1-g, 1/2 at 0-g). Fix one sample at each of the time periods specified. Store.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of the details of cell movement and early morphogenesis in whole embryos at the light and scanning electron microscope level. Analysis of the cell/cell and cell/extracellular matrix interactions at the scanning and transmission electron microscope levels.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g and 0-g 12°C incubator, chamber and equipment for handling toxic fixatives. Storage for fixed materials.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), [cell culture apparatus], [temperature controlled chamber], data storage, chemical fixation capability, refrigeration (4°C), ambient storage.

Experiment Identification Code: DA-d D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Gerald Schatten

Department of Molecular Biology and Zoology
University of Wisconsin
1117 W. Johnson Street
Madison, WI 53706

Critical Question(s) Addressed:

DA-2, DA-4 (See p. A-6)

3. Experiment Title

Microgravity Effects on Sea Urchin Fertilization, Cell Division and Early Development

4. Purpose/Hypothesis

In some vertebrates, gravity effects embryonic development and it is thought that microtubules in the egg during fertilization and the first cell cycle are the transducing cytoskeletal element. This research will determine if fertilization is possible in zero-gravity. The zygotes ability to divide normally will be explored as well the equal and unequal cleavage during early embryogenesis. Finally, calcium deposition in the embryonic skeleton (spicules) will be investigated.

5. Scientific Rationale/Rationale for Microgravity

There are compelling reasons to think that gravity affects development in some vertebrates and this experiment uses a well understood malleable system to determine if microgravity will affect fertilization, division, or early development in sea urchins. This system will not only provide baseline information on the cellular effects of microgravity on fertilization and development, but it also is an inexpensive system in which to test flight hardware for future missions.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Unfertilized sea urchin eggs and sperm will be brought to the space station, and fertilization and early development will be performed in both 0-g and in 1-g conditions. Samples will be fixed at various times during fertilization and development for structural analysis on return.

C. Number and Type of Specimen:

Gametes for the Pacific sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* will be used and specimens will be fixed in at least duplicates with a ground control on the same time line.

D. Measurements/Sample Handling:

Preflight: Prepare fertilization cultures and load fixatives.

In-flight: Mix sperm and eggs to initiate fertilization and development. At various timepoints, fix the specimen within sealed enclosures.

Postflight: Immunocytochemical and electron microscopic analysis will be performed on the fixed and stabilized specimens.

E. Specific Sample Analysis:

In-flight: Determine environmental parameters, e.g. temperature, pH, oxygen.

Postflight: Tubulin, actin, DNA localization, ultrastructural analysis.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

12°C incubator, time lapse video microscope.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), [cell culture apparatus], [temperature controlled chamber], data storage, work area, fluid handling tools, compound microscope, chemical fixation capability, ambient storage.

Experiment Identification Code: DA-e D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Bruce J. Crawford

Faculty of Medicine, Department of Anatomy
University of British Columbia
Vancouver, British Columbia
V6T 1Z3 CANADA

Critical Question(s) Addressed:

DA-2, DB-3, DB-2 (See p. A-6)

3. Experiment Title

Microgravity Effects on Individual Cells Within the Intact Living Embryo

4. Purpose/Hypothesis

To determine how microgravity effects movements and cell/cell and cell/substrate (ECM) interactions of individual living cells within the embryo.

5. Scientific Rationale/Rationale for Microgravity

Studies of the effects of microgravity on movement of individual living cells and their interaction with their substrate in intact embryos should add to our knowledge of how gravity affects the cell/cell and cell/surface interactions which are necessary for normal development.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 60-90 days

B. General Approach Description:

Embryos will be reared to the required stages in microgravity, trapped on a microscope stage and the activities of individual cells recorded using time lapse videomicroscopy. The interaction of the cells with injected FITC labeled ECM components will be followed using fluorescence microscopy and/or a confocal laser scanning microscope.

C. Number and Type of Specimen:

5 adult female and 5 adult male or frozen sperm of the starfish *Pisaster ochraceus*.

D. Measurements/Sample Handling:

Preflight: Obtain and sex adults and preserve sperm and check for activity.

In-flight: Mature and thaw gametes, fertilize and grow cultures to the required stages, trap and record cellular activities and inject labeled ECM components and record cellular activities.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of the details of individual cell movements and their interactions with the various labeled substrate components.

F. Experiment Controls:

In-flight: 1-g controls if possible otherwise none.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Sea water aquaria for maintaining adults, -196°C freezer for maintaining frozen sperm, facilities for culturing embryos in microgravity, microscopes fitted with phase contrast and/or different phase contrast and fluorescence optics and a video recording system. Also required would be a confocal laser scanning microscope and facilities for microinjection of embryos.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), medium aquatic habitat (salt water), [cell culture apparatus], [temperature controlled chamber], data storage, -196°C freezer, fluid handling tools, compound microscope, micromanipulation device, work area.

Experiment Identification Code: DA-f D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Joseph F. Albright

2. Author(s): Dr. Glen Mott

Department of Pathology
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284-7750

Critical Question(s) Addressed:

DA-2, DD-1 (See p. A-6)

3. Experiment Title

Microgravity Influences on Metabolic Programming During Development

4. Purpose/Hypothesis

Microgravity affects plasma hormone levels and developmental cholesterol metabolism post-natally in rats.

5. Scientific Rationale/Rationale for Microgravity

Previous studies with experimental animals have shown that pre- and post-natal hormone administration causes permanent alterations in hormone homeostasis which affects cholesterol metabolism pathways. Long term manned space flight (Mir-Soyez) has produced a 10 fold increase in ACTH which did not return to baseline by 70 days post-flight. Thyroxine levels were also elevated in these cosmonauts. Effects of microgravity on thyroid hormones and corticosteroids were also reported on the Kosmos-1667 flight. Thyroid hormones and corticosteroids affect cholesterol and plasma lipoprotein concentrations. Therefore, microgravity may imprint rates of cholesterol synthesis, uptake and degradation during development and permanently affect lipoprotein concentrations and risk atherosclerosis.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Pregnant rats will be timed to deliver on day 1 or 2 of the flight. Other gravid dams will be timed to deliver after completion of the flight. Blood samples will be drawn from dams or progeny during flight for hormones and lipoproteins. Liver samples will be taken to measure mRNA levels of major regulatory proteins of cholesterol metabolism.

C. Number and Type of Specimen:

Two dozen pregnant rats of each gestational age. Blood samples at -10, -4, 4, 10, and 15 days of flight and 4 days post-flight. Liver removed at sacrifice of dams or progeny and quick frozen (< -80°C) at 15 days in 1/2 of the animal; remainder sacrificed 4 days post-flight.

D. Measurements/Sample Handling:

Preflight: Blood samples.

In-flight: Blood samples, liver.

Postflight: Blood samples, liver.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Thyroid hormones, corticosterone, mRNA for HMG-CoA Reductase, LDL receptor, cholesterol 7 α hydroxylase.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls to in-flight 1-g controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Animal facilities, Low temperature specimen storage.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent birthing habitat, data storage, -70°C freezer, snap/quick freezer, -196°C freezer, fluid handling tools, dissection equipment, work area, dissecting microscope.

Experiment Identification Code: DA-g D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Carey R. Phillips

Department of Biology
Bowdoin College
Brunswick, ME 04011

Critical Question(s) Addressed:

DA-2, DA-3, DB-2, DB-3, DG-3
(See p. A-6)

3. Experiment Title

Role of Gravity in Cytoskeletal Rearrangements During Amphibian Axis Formation

4. Purpose/Hypothesis

Altered gravitational environments may influence the physical location and relationships between various cytoskeletal components. Altered cytoskeletal positions would then effect the repositioning of determinants necessary for correct axis formation.

5. Scientific Rationale/Rationale for Microgravity

Previous studies indicate that cytoplasmic rearrangements are necessary for correct axis formation in amphibians. It has also been shown that both microtubules and actin filaments are involved in the mechanics of relocation. Therefore, a study examining the configuration of the cytoskeleton and specific determinant molecules in a microgravity environment will provide valuable knowledge of cellular mechanisms.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Embryos will be cultured in small cassettes, each of which is temperature controlled. The embryos in the different cassettes would be washed in various solutions. Some cassettes would be processed for antibody staining, some for in situ hybridization and some cassettes would allow embryos to grow to a later developmental stage and then be fixed.

C. Number and Type of Specimen:

Having a number of cassettes would provide flexibility for many types of experiments and many types of analyses. Such a system should provide the opportunity to study any aquatic embryos.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Embryos washed in various solutions.

Postflight: Cassettes are processed for antibody staining and in situ hybridization.

E. Specific Sample Analysis:

In-flight: None.

Postflight: In situ hybridization, antibody staining and general histology.

F. Experiment Controls:

In-flight: Cassettes on an inflight centrifuge.

Ground: Embryos grown in cassettes under similar conditions in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Computerized unit containing cassettes and the different reservoirs of solutions.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (fresh water), [cell culture apparatus], (temperature controlled chamber), data storage, chemical fixation capability.

Experiment Identification Code: DA-h D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Anton W. Neff

Medical Sciences Program
Indiana University (Medicine)
Bloomington, IN 47405

Critical Question(s) Addressed:

DA-2, DA-6, DB-1, DG-1 (See p. A-6)

3. Experiment Title

Analysis of Amphibian Pregastrulation and Gastrulation Movements in Microgravity

4. Purpose/Hypothesis

Pregastrulation and gastrulation movements are affected by microgravity exposure.

5. Scientific Rationale/Rationale for Microgravity

Ground based microgravity simulation by clinostat rotation has shown that the morphology of microgravity reared midblastula embryos and gastrulas is affected. The shape and location of the blastocoele and the cellularity of the blastocoele roof is affected, as well as the location of the initiation site and the shape of the dorsal lip of the blastopore (involution site). Pregastrulation and gastrulation cell movements may be responsible, in part, for these microgravity induce blastula and gastrula dismorphogenesis.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and 3

B. General Approach Description:

Phase 1 flights: Analyze pregastrulation and gastrulation movements indirectly on a fixed series of space flown *Xenopus* embryos (fertilization through gastrulation). Analysis will be conducted on the ground utilizing light and scanning electron microscopy.

Phase 3 flights: Observe the surface pregastrulation and gastrulation movements as well as neurulation and migration dynamics of neurons in real time. This would require having video cameras and the appropriate microscopes set up in the space station to record movements directly. These experiments would involve setting up a microinjection apparatus in space for the injection of cell lineage markers into selected cells and then monitoring the movements of those cells utilizing high sensitivity video recordings (fluorescence).

C. Number and Type of Specimen:

Live amphibians in space station for source of eggs and sperm. Species: *Xenopus laevis*. All experiments will be conducted on embryos between fertilization and neurulation. It would be preferable for Phase 3 experiments to be timed with the return of a shuttle from station, so control eggs and sperm from the same spawning can be returned to Earth for synchronous control experiments.

D. Measurements/Sample Handling:

Preflight: Phase 1: None.
Phase 3: None.

In-flight: Phase 1: Fixation and storage of embryos at various stages.
Phase 3: Fertilization; observation of pregastrulation and gastrulation movements in real time; microinjection of cell lineage markers and monitoring of cell movements.

Postflight: Phase 1: None.
Phase 3: None.

E. Specific Sample Analysis:

In-flight: Phase 1: None.
Phase 3: Analysis of pregastrulation, gastrulation, neurulation, and migration dynamics of neurons real time utilizing high sensitivity video recordings.
Postflight: Phase 1: Analyze pregastrulation and gastrulation movements indirectly on a fixed series of space flown *Xenopus* embryos.
Phase 3: None.

F. Experiment Controls:

In-flight: Phase 1: Space-centrifuged embryos (1-g) from the same spawning.
Phase 3: None.

Ground: Phase 1: Synchronous ground based controls with embryos from the same spawning.
Phase 3: Synchronous ground-based experiments with embryos from the same spawning.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

UF: Amphibian maintenance unit, dissecting microscope, facility for manipulation and maintaining embryos, centrifuge that can produce one times gravity.

PMC: Same as above plus micromanipulator, microinjection apparatus, fluorescent microscope, high sensitivity video camera and image recording equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Phase 1: Small aquatic habitat (fresh water), medium aquatic habitat (fresh water), [cell culture apparatus], [temperature controlled chamber], data storage, fluid handling tools, dissecting microscope, chemical fixation capability, work area, ambient storage.

Phase 3: Same as above plus: micromanipulation device, compound microscope.

Experiment Identification Code: DA-i

D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Carey R. Phillips

Department of Biology
Bowdoin College
Brunswick, ME 04011

Critical Question(s) Addressed:

DA-2, DB-2, DB-3, DG-1, DG-3
(See p. A-6)

3. Experiment Title

Regulation of Developmental Processes When Disturbed by a Microgravity Environment and Assayed at the Molecular Level

4. Purpose/Hypothesis

There are a number of animals which can undergo a certain amount of regulation for developmental abnormalities when subjected to environmental perturbations.

5. Scientific Rationale/Rationale for Microgravity

If an animal, or plant, undergoes a developmental regulatory process in response to an environmental stress, the final morphology of the organism may appear quite normal. However, it is of interest to understand when and how organisms are regulating at the molecular level. These studies will provide information about the range of harmful environmental stresses and the developmental time periods when organisms are most susceptible to these stresses. Molecular probes will be used to assay for changes in developmental process or developmental plan. These same molecular probes can be used to study the mechanisms for the process of regulation.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and Phase 3 (30-90 days)

B. General Approach Description:

Fertilize and develop flies (and amphibians) in space for varying periods of development. Fix and return to Earth based laboratory for analysis.

C. Number and Type of Specimen:

Currently, the best species to work with is *Drosophila* and *Xenopus*. Several samples at each developmental stage (5) would be needed.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Fertilize flies (and amphibians). Fix specimens at specified stages.

Postflight:

E. Specific Sample Analysis:

In-flight: None.

Postflight: Northern analysis and in situ hybridization.

F. Experiment Controls:

In-flight: Embryos grown in a space centrifuge.

Ground: Embryos grown on Earth.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Fixation capability, *Drosophila* and *Xenopus* embryo growth chambers.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, small aquatic habitat (fresh water), medium aquatic habitat (fresh water), [cell culture apparatus], [temperature controlled chamber], data storage, fluid handling tools, chemical fixation capability, work area, ambient storage.

Experiment Identification Code: DA-j

D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Gerald Schatten

2. Author(s): Dr. Stephen A. Stricker

Department of Biology
University of New Mexico
Albuquerque, NM 87131

Critical Question(s) Addressed:

DA-3 (See p. A-6)

3. Experiment Title

Microgravity Effects on the Nucleolus During Oocyte Maturation

4. Purpose/Hypothesis

The nucleolus, which must disassemble and reassemble during oocyte maturation, is believed to be gravity sensitive. Thus we predict that the nucleoli of oocytes triggered to undergo maturation in microgravity will exhibit structural, biochemical, and/or functional changes that differ from those observed in Earth-based counterparts.

5. Scientific Rationale/Rationale for Microgravity

During normal oocyte maturation in starfish, the nucleolus must undergo dramatic changes in structure and function for development to proceed. Recently, nucleoli have been hypothesized as functioning as gravity-sensitive "nuclear statoliths" (Moroz, P. 1984, J. Theor. Biol., 107: 303-20) that can modulate cell division. This study will analyze the possible effects of microgravity on nucleoli and the patterns of oocyte maturation.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Oocytes will be treated with hormone to trigger oocyte maturation at both 0-g and 1-g, and samples from each culture condition will be fixed at 10 minute intervals for up to 100 minutes. The fixed samples will then be processed post-flight for electron microscopy, confocal immunofluorescence microscopy, immunoblotting, and biochemical methods to assess microgravity-induced changes in nucleolar structure and function during oocyte maturation, as compared to those observed in ground-based controls.

C. Number and Type of Specimen:

Ripe adult female starfish (*Pisaster ochraceus* or *Asterina miniata*) will be used. Four replicates of each fixation (11 fixation times) will be prepared for both culture conditions (i.e., total samples = 88)

D. Measurements/Sample Handling:

Preflight: Prepare oocytes.

In-flight: Hormone treatment and fixations.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Electron microscopy; confocal immunofluorescence; 1 or 2D gels; immunoblotting of nucleolar components.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Refrigerated (15 +/-1°C) incubator, 1-g refrigerated (15 +/-1°C) incubator, eighty 100-ml sample bags for adding hormone and fixative (ala Spangenberg models).

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), [cell culture apparatus], [temperature controlled chamber], data storage, chemical fixation capability, ambient storage.

Experiment Identification Code: DA-k D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-Q)

Critical Question(s) Addressed:

DA-4, DD-1 (See p. A-6)

3. Experiment Title

Fecundity of Insects in Space

4. Purpose/Hypothesis

Weightlessness may affect the fecundity of female insects and the viability of embryos.

5. Scientific Rationale/Rationale for Microgravity

Previous work in altered gravity suggests that these particular parameters may be affected by the stresses of the spaceflight environment.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

The number of eggs deposited per female will be recorded and viability will be determined. Behavior will be observed.

C. Number and Type of Specimen:

20 male, 30 female fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Record number of eggs deposited per female. Determine viability. Observe behavior for changes in postural control.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Controls as per flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Insect habitat, small mass measure, dissecting microscope, glovebox.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage, dissection equipment, work area.

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Gregory A. Nelson

Space Biological Sciences Group, MS 89-2
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, CA 91109

Critical Question(s) Addressed:

DA-4 (See p. A-6)

3. Experiment Title

Acrosome Reactions and Polymerization of Motility Proteins in Microgravity

4. Purpose/Hypothesis

The balance of forces involved in rapid polymerization of motility proteins (actin complex) in invertebrate acrosome reactions will be perturbed in microgravity. In addition, the ionic movements at and near the cell membrane, which control the initiation of the acrosome reaction, will take place under strictly diffusion limited conditions. It is hypothesized that these features of gravity unloading will lead to alterations in the rate and extent of the acrosome reaction which represents a large scale *in vivo* protein polymerization event.

5. Scientific Rationale/Rationale for Microgravity

Self assembly and phase change processes of biological macromolecules are regulated by concentrations of co-factor molecules, ionic environment and weak forces involved in ligand-receptor associations, allosteric changes and noncovalent binding that occurs between recognition surfaces of interacting molecules. Under conditions where such associations of molecules occur in an amplified process, small alterations in the rates and extents of association should be measurable and small perturbations in environment should translate to easily detectable changes in the assembly of large structures. Microgravity effectively eliminates density driven convection thus the forces which dominate in microgravity will be of the same order of magnitude as that of polymerization. This raises the possibility that small differences in local environments will alter the kinetics of polymerization, ligand-receptor binding and ionic movements.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Sperm of several marine invertebrates such as sea squirts (e.g. *Ciona*) and sea cucumbers (e.g. *Thyone*) react to the presence of proteases, high pH and specific substances in the gelatinous coatings of oocytes (egg jelly) which trigger the so-called acrosome reaction. The acrosome reaction begins with ligand-receptor binding at the cell membrane which results in a membrane fusion event that releases the contents of a large vesicle (acrosome). The contents consist of the motility protein, actin, plus accessory proteins that rapidly polymerize into a long filament which mechanically aids in the penetration of the jelly coat of an egg by the reacting sperm. By mixing freshly harvested eggs (or purified jelly) and sperm it is possible to generate many thousands of acrosome reactions which can be visualized in real time or as fixed specimens for the rate and extent of *in vivo* polymerization. An advanced version of the experiment might also explore fertilization and early development.

C. Number and Type of Specimen:

Several male sea cucumbers and extracts containing egg jelly or activator. Alternatively several hermaphrodite sea squirts. Animals maintained in sea water for isolation of at least 20 1 ml samples of fresh sperm at 10^4 per milliliter.

D. Measurements/Sample Handling:

- Preflight: Select individual animals of appropriate sex and age. Induce gametogenesis by hormonal treatment. From eggs, isolate and purify active egg jelly or prepare chemical activator in stable form at known concentration. Load sample containers.
- In-flight: Incubate samples at 12-20°C. Mix samples according to predetermined timeline followed by fixation, resulting in a series of reactions as functions of time. Some samples are viewed by videomicroscopy to measure rate of elongation of acrosomal filaments.
- Postflight: Reaction mixtures are divided into aliquots for light microscopy using phase-contrast and immunofluorescence of anti-actin antibodies or the filamentous actin probe rhodamine-phalloidin. Electron microscopy is performed to characterize filament structure.

E. Specific Sample Analysis:

- In-flight: Recording of acrosome reactions by videomicroscopy to measure kinetics of polymerization. Mixing and fixing of other samples at defined times. Sample analysis is postponed until landing.
- Postflight: Measure elongation rates of acrosomal filaments recorded on videotape. Prepare fixed samples for light and immunofluorescence microscopy and electron microscopy as needed.

F. Experiment Controls:

- In-flight: In-flight centrifuge control should be performed with same lot of materials. The expected difference is the presence of convection cells and possibly some isothermal settling.
- Ground: Parallel ground controls with same lots should be performed.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Incubation at 10-20°C with and without 1-g centrifuge. Crew isolation of gametes followed by activation and fixation of samples manually or automatically. Videomicroscopy for ≤ 5 minutes per sample. Stow/unstow jelly or activator in cold at -20°C.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), medium aquatic habitat (salt water), [cell culture apparatus], [temperature controlled chamber], data storage, fluid handling tools, compound microscope, -20°C freezer, work area, chemical fixation capability, ambient storage.

Experiment Identification Code: DA-m D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. David G. Capco

Molecular and Cell Biology Program
Department of Zoology
Arizona State University
Tempe, AZ 85287-1501

Critical Question(s) Addressed:

DA-2, DA-6, DG-1 (See p. A-6)

3. Experiment Title

Gravity-induced Effects on the Establishment of First Cellular Polarity of Mammalian Embryos/Effects on Signal Transduction Pathways

4. Purpose/Hypothesis

Zero-gravity will alter the timing of signal transduction events and consequently alter the establishment of first polarity in mammalian embryos.

5. Scientific Rationale/Rationale for Microgravity

Mammalian embryogenesis is unique in requiring establishment of cellular polarity between the S-32 cell stages of embryogenesis although the molecular basis for the initiation of this polarity remains undetermined. In some developing systems gravity has a central role in the establishment of embryonic polarity (i.e. axis formation). Gravity may also function in the initiation of polarity in mammalian embryos. It is important to determine the effects of zero-gravity on initiation of embryonic polarity since in humans the appropriate stage of development would be reached before a woman would be aware that conception has occurred.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Eggs and embryos of the Golden Syrian hamster will be obtained in flight, cultured for the appropriate time and cytochemically fixed and then stored for the return flight. Hamster eggs and embryos contain specialized cytoskeletal elements referred to as sheets which serve as endogenous markers for the establishment of cellular polarity and for some signal transduction pathways. These cytoskeletal elements will be analyzed postflight.

C. Number and Type of Specimen:

Ten female hamsters will be required to provide for triplicate experiments.

D. Measurements/Sample Handling:

Preflight: Female hamsters will be injected with appropriate hormones to enhance reproductive cycle. Some male hamsters will be used to provide sperm, while other male hamsters will be mated with primed female hamsters.

In-flight: Collect eggs or embryos, mix with sperm where appropriate, culture in incubator, apply cytochemical fixative at specific time intervals.

Postflight: Examine for organization of specialized cytoskeletal elements using a confocal microscope, or after processing for electron microscopy.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Compare orientation of sheets at time intervals with controls maintained at 1-g. Stain with Con. A to confirm timing of polarity establishment. Correlate spatial pattern of sheets with level of activity of protein kinase C established in control experiments. (Access to confocal and transmission electron microscope required.)

F. Experiment Controls:

In-flight: 1-g controls.

Ground: 1-g controls will parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ incubator, 1-g CO₂ cell incubator.

B. GBF Hardware Capabilities Required (on Orbit)

(Cell culture apparatus), rodent weanling to adult habitat, [temperature controlled chamber], data storage, work area, chemical fixation capability, fluid handling tools, ambient storage.

Experiment Identification Code: DA-n D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Gregory A. Nelson

Space Biological Sciences Group, MS 89-2
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, CA 91109

Critical Question(s) Addressed:

DG-5, DA-6, DD-1 (See p. A-6)

3. Experiment Title

Nematode Development and Genome Stability in LEO Environment

4. Purpose/Hypothesis

Gravity may be required for establishing asymmetry in development and for normal chromosome mechanics during mitosis and meiosis. Furthermore, exposure to low dose, low dose rate protons and cosmic rays in Earth orbit may efficiently induce genetic changes whose detection and repair may be perturbed under microgravity.

5. Scientific Rationale/Rationale for Microgravity

Under microgravity, dense subcellular structures such as chromosomes will not sediment nor will slight deformations of tissue and organs occur which could modulate surface ion channels (e.g. stretch sensitive ion channels) and receptors. These subtle changes when amplified by biochemical and cytoskeletal reactions may be sufficient to establish and maintain polarity and alignment of structures during development. The presence of naturally occurring high LET radiation in space is known to have radiobiological consequences at the genetic and developmental level. Together these environmental effects present in the Space Station orbital space may interact either acutely or over several generations to disrupt development and genome stability.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

The nematode *C. elegans* undergoes a precise developmental program in which individual cell fates have been described and related to precise cell lineage patterns. Genetically marked strains can be used to track generations, monitor chromosome segregation, recombination and mutation. Serial transfers of worms over multiple generations would allow quantitation of developmental errors and detection of impaired chromosome mechanics and mutation. Worms can be forced in and out of a dormant developmental state using pheromone treatment or frozen and fixed for transport to and from orbit and analysis on the ground.

C. Number and Type of Specimen:

Nematodes of wild type and mutant strains which monitor segregation, recombination and mutation in specific structural genes or essential genes in balanced regions are cultured in agar lined tubes seeded with bacteria of approximately 5 ml volume. Each tube would receive 1 to 10 animals which would reproduce over approximately 5 days to about 1000 offspring. Approximately 4 mutant strains would be used in triplicate both at 1 g and μ g.

D. Measurements/Sample Handling:

- Preflight: Mutant strains of worms, tube cultures without bacteria, bacterial suspensions, dauer larva pheromone, fixative and cryoprotectant solutions prepared. Spontaneous mutation rates and developmental errors would be measured in worm cultures.
- In-flight: Samples placed in incubators and refrigerators. Subcultures and bacterial food transferred to tubes at regular intervals. After each 5-day period, cultures are treated with pheromone to force animals into dauer larva state or split into subsets for freezing or fixation.
- Postflight: Frozen and dormant larvae are reactivated by incubation on bacteria at 20°C. Proportions of mutant phenotypes are scored in population which progressed various numbers of generation in orbit. Fixed samples are scored for anatomical features.

E. Specific Sample Analysis:

- In-flight: Sample analysis is postponed until landing.
- Postflight: Selection for mutants in the *unc-22* gene and in essential genes balanced by the *eT1* translocation. Fixed samples are analyzed for cellular anatomy. Gonad symmetry, cell number and position in several organs are scored as well as the frequency of anaphase bridges in intestinal cells. The occurrence of recombination phenotypes is measured.

F. Experiment Controls:

- In-flight: In-flight centrifuge controls performed with sibling cultures.
- Ground: Parallel ground controls with sibling subcultures should be performed. X-ray or gamma ray exposures would be used to mimic some aspects of radiation exposure.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Incubation at 15 to 20°C with and without 1-g centrifuge and 4°C storage for bacteria. Crew to conduct fluid addition and removal from tubes. This includes animal transfers, pheromone addition and fixation/freezing.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage, refrigeration (4°C), chemical fixation capability, -70°C freezer, work area, ambient storage.

Experiment Identification Code: DA-o D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. D.C. Holley & Dr. R.E. Stecker

Department of Biological Sciences
San Jose State University
1 Washington Square
San Jose, CA 95192-0100

Critical Question(s) Addressed:

DA-4, DA-8, DC-1, DC-3, DD-1, DE-2
(See p. A-6)

3. Experiment Title

Insect (Silverfish) Reproduction and Development in Microgravity

4. Purpose/Hypothesis

We do not fully understand the effects of gravity on normal growth and development signals in complex multi-cellular animals. In addition, the role of gravity in normal reproductive behavior is not well defined. This study will determine the effects of microgravity on reproductive behavior, fertilization, and growth and development of three genera of silverfish.

5. Scientific Rationale/Rationale for Microgravity

It is important to establish a model to study microgravity effects on reproductive behavior, growth, and development of a complex multi-cellular animal. Using animals from three genera will allow one of the first comparative assessments of the effects of microgravity. The silverfish is a hardy insect that is often the first to occupy newly available habitat (e.g. a newly constructed house). They have a wide temperature tolerance, can reproduce in the dark in the absence of a photo period, do not require free water (they utilize metabolic water), have minimal feed requirements being able to thrive for months on a small (< 0.25 gram) piece of bread, and require minimal space. Silverfish housed together do not show cannibalistic behavior exhibited by other insects. Silverfish are ametabolous (they do not undergo complete metamorphosis), therefore, they would be a good contrast to the much studied *Drosophila* which have complete metamorphosis.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2: 180 days

B. General Approach Description:

The insects (various mating pairs, instars, and the eggs) will be exposed to microgravity for up to 180 days. The insect habitat will not require any crew intervention while on orbit. All analysis and measurements will be done post flight in the investigators' laboratories.

C. Number and Type of Specimen:

- *Lepisma saccharina*, 75 including egg clutches, males/females, and representatives of the five instars.
- *Ctenolepisma Longicaudata*, 75 including egg clutches, males/females, and representatives of the five instars.
- *Leucolepisma arenaria*, 75 including egg clutches, males/females, and representatives of the five instars.

D. Measurements/Sample Handling:

Preflight: None

In-flight: None

Postflight: Body size; weight; gamete histology; sex determination of hatched eggs and offspring conceived on orbit; stadia duration (duration of instars); other biochemical/physiological parameters (to be determined) to assess normal growth and development in microgravity.

E. Specific Sample Analysis:

In-flight: None

Postflight: None

F. Experiment Controls:

In-flight: None

Ground: Same as in-flight, i.e., synchronous controls

7. Hardware Requirements

A. Minimum on Orbit Requirements

Multi-compartment insect habitat containing approximately 225 individual chambers measuring approximately 10x10x40 mm (4mL). Overall habitat dimensions and shape are not critical. It would require only approx. 2-3 liters in volume. The habitat would require pressurization and rough passive temperature control. It would not require light control or sound attenuation.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage.

Experiment Identification Code: DA-p D = developmental; A = early developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Bruce Hammock

2. Author(s): William T. Sullivan

Sinsheimer Lab
Department of Biology
University of California
Santa Cruz, CA 95064

Critical Question(s) Addressed:

DA-6, DA-7

3. Experiment Title

The Effects of Microgravity on Early Drosophila Development

4. Purpose/Hypothesis

To study whether microgravity disrupts any of the following processes:

- nuclear division
- the synchrony of nuclear division
- the pattern and timing of nuclear migration
- the timing and expression pattern of the Homeobox containing segmentation gene *fushi tarazu*.

5. Scientific Rationale/Rationale for Microgravity

Drosophila is an excellent system to study the effect of microgravity on both early metazoan development and cell division. The initial divisions are rapid (10 minutes), synchronous and undergo a developmental controlled pattern of migrations. In addition, specific genes such as *fushi tarazu* which are fundamentally involved in the primary determinative events of the embryo become expressed in very specific patterns at this time.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1; 10 to 12 days

B. General Approach Description:

A population of approximately 500 wild-type Drosophila (in two half pint bottles) is allowed to lay eggs for 4 hours at 25°C. The eggs are collected, dechorionated with bleach, and fixed in formaldehyde by first incubating the embryos in heptane for 30 seconds. The embryos will be stored in methanol and immunofluorescent analysis will be performed upon return to ground. A control set of embryos will be collected and fixed on earth at the same time as the collections are being performed on-orbit.

Immunofluorescent analysis on a confocal laser scanning microscope will be used to determine nuclear distribution patterns and the expression pattern of *fushi tarazu*.

C. Number and Type of Specimen:

Fruit fly, *Drosophila melanogaster*, 500 adults

D. Measurements/Sample Handling:

Preflight: None

In-flight: Collect eggs. Dechorionate eggs with bleach. Incubate embryos for 30 seconds in heptane, fix in formaldehyde and store in methanol.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Determine nuclear distribution patterns and expression pattern of *fushi tarazu* by immunofluorescent analysis on a confocal laser scanning microscope.

F. Experiment Controls:

In-flight: None

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Egg collection set-up, fixation capability, dissecting microscope-useful.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage, dissection equipment, work area, chemical fixation capability, ambient storage.

Experiment Identification Code: DB-a

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Michael L. Wiederhold

Department of OHNS
University of Texas Health Science Center
San Antonio, Texas 78284-7777

Critical Question(s) Addressed:

DA-2, DB-1, DC-4, DG-1, DG-4
(See p. A-6)

3. Experiment Title

Development of Gravity-sensing Organs in Microgravity

4. Purpose/Hypothesis

During development of the otoliths (masses upon which gravity acts in gravity-sensing organs), otolith weight is regulated to be optimal for the species and developmental stage.

5. Scientific Rationale/Rationale for Microgravity

If otolith weight is controlled, otoliths should be larger in microgravity and smaller in hypergravity, compared to 1-g reared control animals. We are currently performing studies on the early development of the gravity-sensing organs in several species, including the marine mollusc *Aplysia californica* and the Japanese red-bellied newt, *Cynops pyrrhogaster*, a fresh water amphibian. *Aplysia* reared on a 2-g centrifuge produce statoliths smaller than control (1-g) specimens, relative to total body size. The newts will be flown on IML-2 to study otolith and sensory epithelium development in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 (and Phase 2 or 3, 30-180 days)

B. General Approach Description:

Adult specimens will be maintained at 0-g. If adult *Aplysia* will mate on orbit, egg strands will be laid the day after mating and develop for approximately 10 days before hatching. Specimens will be retrieved at various stages and fixed for anatomical processing after return to Earth. Adult female newts, "premated" in the field, but in which eggs are not fertilized, will be injected with hormone on orbit to induce egg shedding, fertilization and embryonic development. Eggs at various stages will be harvested for anatomical and physiological study. With 100+ day missions (Phase 2 or 3), larvae will develop to metamorphosis, at which stage the statocyst goes through a major transformation, which is believed to be dependent on gravity. Similarly, newts will develop to the stage at which orienting and vestibulo-ocular reflexes can be tested. Centrifugation will test whether any anomalies in the vestibular system of specimens reared in micro-g can be avoided or rectified by exposure to 1-g or hyper-g.

C. Number and Type of Specimen:

6 adult *Aplysia californica*, 100-250 grams.

6 adult newts, *Cynops pyrrhogaster*, collected in the fall in Japan and three of which will have been injected with hormone two days before launch.

D. Measurements/Sample Handling:

Preflight: Inject 3 female newts with 200 IU human chorionic gonadotropin 2 days before launch.

In-flight: Maintain both species in appropriate aquatic animal facility, one set at 0-g, the other on a 1-g centrifuge. Selected specimens harvested and fixed while in orbit. Inject 3 female newts with 200 IU human chorionic gonadotropin in orbit.

Postflight: Section specimens for analysis.

E. Specific Sample Analysis:

In-flight: Observe behavior of adults: swimming, orienting behavior.

Postflight: Analyze sectioned specimens using computer-based image analysis, to determine otolith and statolith volume and form of sensory epithelium. Study physiology of gravity-sensing organs developed in microgravity.

F. Experiment Controls:

In-flight: 1-g centrifuge control.

Ground: Either synchronous or delayed experiments paralleled to in-flight experiments. (On Earth centrifuge would produce net g-field of 1.4-g, which would allow study of the effects of hypergravity, with the same rotational stimulus used to produce 1-g on orbit.)

7. Hardware Requirements

A. Minimum on Orbit Requirements

Aquarium system for maintaining adult specimens, volume approximately 5 liters. This does not need to have a 1-g control on orbit. 1A 1-g control centrifuge system to maintain developing specimens. This only needs 100 to 200 ml compartment to contain aerated water. All systems should be able to tolerate either fresh or salt water to allow different species to be studied.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (fresh water / salt water), medium aquatic habitat (fresh water / salt water), data storage, fluid handling tools, chemical fixation capability, work area, ambient storage.

Experiment Identification Code: DB-b D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Timothy A. Jones

College of Dentistry
U.M.N.C.
Lincoln, NE 68583-0740

Critical Question(s) Addressed:

DC-4, DB-1 (See p. A-6)

3. Experiment Title

Effects of Weightlessness on Vestibular Development, Phase 1

4. Purpose/Hypothesis

This experiment will test the hypothesis that depriving embryos of a steady gravitational field will alter developmental patterns in the vestibular system.

5. Scientific Rationale/Rationale for Microgravity

All life evolved under the influence of Earth's gravitational field. Therefore gravity sensors have always developed in the presence of an adequate sensory stimulus and gravity may play some role in guiding vestibular ontogeny. Sensory deprivation in other modalities is known to alter development (e.g. vision, audition). The microgravity environment of space presents the unique possibility of depriving developing embryos of some forms of vestibular sensory stimuli that are normally found on Earth at 1.0-g. Will vestibular systems develop normally without gravity?

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 7-16 days

B. General Approach Description:

Avian embryos will be exposed to gravitational fields between 0-g and 1-g. The period of exposure will be systematically varied to aid in the identification of periods of critical sensitivity. During Phase 1 flights, it will not be possible to expose embryos to microgravity throughout the normal incubation period (i.e., 21 days) and therefore, those experiments will be undertaken in Phases 2 and 3. Vestibular thresholds and activation latencies will be measured to evaluate changes in vestibular ontogeny. Tissues will be fixed and processed to evaluate morphological changes.

C. Number and Type of Specimen:

Incubators should accommodate 30 to 50 fertilized eggs.

D. Measurements/Sample Handling:

Preflight: Fertilized chicken eggs will be cooled to arrest development.

In-flight: Incubation temperatures will be raised at the appropriate time to initiate development in space.

Postflight: Functional and morphological testing will be carried out on embryos or hatchlings upon return to Earth.

E. Specific Sample Analysis:

In-flight: Automatic incubation timing. Automatic fixation.

Postflight: Vestibular response thresholds and activation latencies will be determined in embryos and hatchlings. Anatomical characteristics of vestibular structures will also be studied.

F. Experiment Controls:

In-flight: 1-g centrifuge controls.

Ground: 1) Synchronous ground controls undergoing simulated lift-off and landing conditions of vibration and g-force. 2) normal laboratory controls and 3) animals exposed to hypergravitational fields (complementary experiments).

7. Hardware Requirements

A. Minimum on Orbit Requirements

Avian incubator with the following capabilities: 1) programmable temperature for automatic cooling and warming of embryos in flight 2) automatic injection of fixatives 3) 1 g centrifugation of fertilized eggs.

B. GBF Hardware Capabilities Required (on Orbit)

Avian egg incubator, avian hatchling habitat, data storage, chemical fixation capability.

Experiment Identification Code: DB-c

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Timothy A. Jones

College of Dentistry

U.M.N.C.

Lincoln, NE 68583-0740

Critical Question(s) Addressed:

DC-4, DB-1 (See p. A-6)

3. Experiment Title

Effects of Weightlessness on Vestibular Development, Phase 2

4. Purpose/Hypothesis

This experiment will test the hypothesis that depriving embryos of a steady gravitational field will alter developmental patterns in the vestibular system.

5. Scientific Rationale/Rationale for Microgravity

All life evolved under the influence of Earth's gravitational field. Therefore gravity sensors have always developed in the presence of an adequate sensory stimulus and gravity may play some role in guiding vestibular ontogeny. Sensory deprivation in other modalities is known to alter development (e.g. vision, audition). The microgravity environment of space presents the unique possibility of depriving developing embryos of some forms of vestibular sensory stimuli that are normally found on Earth at 1.0-g. Will vestibular systems develop normally without gravity?

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2, 30 days

B. General Approach Description:

Avian embryos will be exposed to microgravitational fields between 0-g and 1-g. The period of exposure will be throughout the normal incubation period (i.e. approximately 21 days). Development will be arrested for 10 days in flight and then activated automatically and continue until return to Earth. Animals will return to Earth on the day of hatch. Vestibular thresholds and activation latencies will be measured to evaluate changes in vestibular ontogeny. Tissues will be fixed and processed to evaluate the morphological changes.

C. Number and Type of Specimen:

Incubators should accommodate 30 to 50 fertilized eggs.

D. Measurements/Sample Handling:

Preflight: Fertilized chicken eggs will be cooled to arrest development.

In-flight: Incubation temperatures will be raised at the appropriate time to initiate development in space.

Postflight: Functional and morphological testing will be carried out on embryos or hatchlings. Anatomical characteristics of vestibular structures will also be studied.

E. Specific Sample Analysis:

In-flight: Automatic incubation timing.

Postflight: Vestibular response thresholds and activation latencies will be determined in embryos and hatchlings. Anatomical characteristics of vestibular structures will also be studied.

F. Experiment Controls:

In-flight: 1-g centrifuge controls.

Ground: 1) Synchronous ground controls undergoing simulated lift-off and landing conditions of vibration and g force, 2) normal laboratory controls, and 3) animals exposed to hypergravitational fields (complementary experimental animals).

7. Hardware Requirements

A. Minimum on Orbit Requirements

- 1) Programmable temperature for automatic cooling and warming of embryos in flight
- 2) 1.0 g centrifugation of fertilized eggs.

B. GBF Hardware Capabilities Required (on Orbit)

Avian egg incubator, data storage, refrigeration (4°C).

Experiment Identification Code: DB-d D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. Ernest J. Peck

Science and Mathematics, UNLV
4505 South Maryland Pkwy
Las Vegas, NV 89154-4001

Critical Question(s) Addressed:

DB-1, DB-3, CA-2, CB-3 (See p. A-6)

3. Experiment Title

Microgravity Effects on Synaptic Activity

4. Purpose/Hypothesis

Recycling of synaptic vesicles will differ under microgravity conditions when compared to 1-g controls.

5. Scientific Rationale/Rationale for Microgravity

The secretion of neurotransmitters from nerve terminals involves exocytosis from synaptic vesicles, with vesicles subsequently recycled via recapture and refilling. The details of this process are not understood but are thought to involve a filamentous network composed of synapsin I (Altorta et al, Neuroscience, 24: 593, 1988) and molecular motors involving cytoskeleton. Microgravity may alter microtubule assemblies. A study of the effects of microgravity on synaptic vesicle recycling will provide essential knowledge of neuronal function in microgravity. Such a study would serve as precursor to a much larger question concerning synaptogenesis under conditions of microgravity (see E. Peck, Microgravity Effects on Synaptogenesis (reference experiment DB-e)).

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Frogs will be maintained under 0-g and 1-g conditions (optimally, both would be in flight to limit variables; however, 1-g controls could be done on Earth) and sacrificed at intervals over 14 days for acute cutaneous pectoris nerve-muscle preparations. The fluorescence microscopy/video imaging methodology of Betz and Bewick (Science, 255: 200, 1992) would be employed for optical monitoring of synaptic activity in these nerve-muscle complexes. Fluorescent dyes label motor nerve terminals under stimulation so that dye uptake by synaptic vesicles can serve as a monitor of vesicular recycling and synaptic activity.

C. Number and Type of Specimen:

9 -12 frogs would be used under each condition (0-g and 1-g) to insure adequate sampling at 3 points in flight duration (1, 7, 14 days). Each muscle prep allows the sampling of multiple neuromuscular junctions. About 4 neuromuscular junctions per muscle prep, with duplicate preps per time point and three time points (1, 7, 14 days) would result in 24 samples per condition or 48 samples for both 0-g and 1-g.

D. Measurements/Sample Handling:

Preflight: Prepare stock solutions, acclimate frogs.

In-flight: Dissect neuromuscular junctions, expose to dyes, stimulate and record with fluorescence videomicroscope.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Computer analysis of video images; characterization of recycling patterns.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g. These can be performed repetitively prior to flight to test all equipment as well.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Animal containment facilities for frogs (0 and 1-g if possible) these could be as simple as plexiglass boxes with moss for communal containment; dissection equipment, fluorescent microscope with water and oil-immersion lenses, filters, video camera, data storage device.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (fresh water) or Temperature controlled chamber, data storage, fluid handling tools, dissection equipment, compound microscope, dissecting microscope, work area.

Experiment Identification Code: DB-e

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Bruce Umminger

2. Author(s): Dr. Ernest J. Peck

Science and Mathematics, UNLV
4505 South Maryland Pkwy
Las Vegas, NV 89154-4001

Critical Question(s) Addressed:

DB-1, DB-2, CA-2, CB-3 (See p. A-6)

3. Experiment Title

Microgravity Effects on Synaptogenesis

4. Purpose/Hypothesis

Synaptogenesis will differ under microgravity conditions when compared to 1-g controls.

5. Scientific Rationale/Rationale for Microgravity

Synaptogenesis, the development of synapses between neurons or between nerves and muscle, requires the coordinate control of cytoskeletal and membranous elements of two independent cellular entities. Although the details of this process are not well understood, synaptogenesis is the subject of intense investigation in the field of developmental neurobiology. Interest for this project stems from the role of cytoskeletal elements in the coordinate control of the process of synapse formation. What role does gravity play in coordination of this functional union of neurons? The fluorescence microscopy assay (see E. Peck, Microgravity Effects on Synaptic Activity (reference experiment DB-d)) could be used to ask whether functional synapses had been formed in neuronal cultures *in vitro*. Thus the scoring of functional synapses in culture in microgravity vs. those formed in 1-g could address whether microgravity interfered in the process.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (90 days)

B. General Approach Description:

Neuronal cultures will be maintained under 0-g and 1-g conditions (optimally, both would be in flight to limit variables; however, 1-g controls could be done on Earth) and exposed at intervals over 90 days to fluorescent dyes for the assessment of synaptic activity (as measured by synaptic recycling). The fluorescence microscopy/video imaging methodology of Betz and Bewick (*Science*, 255: 200, 1992) would be employed for optical monitoring of synaptic activity in these neuronal cultures.

C. Number and Type of Specimen:

Replicate plates will be exposed to fluorescent dyes at intervals of 2-4 days over a three week period. Experiments will be repeated at intervals of 21 days to assess the effect of long-term exposure to 0-g on synaptogenesis. Seven time points per three week study with replication, two conditions (0-g and 1-g), and four three week studies would result in 112 samples.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks, stock solutions.

In-flight: Set-up cultures, expose samples to fluorescent dyes, stimulate, and record with fluorescence videomicroscope at 2-4 day intervals.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Computer analysis of video images; assessment of recycling.

F. Experiment Controls:

In-flight: 1-g controls

Ground: Controls will parallel in-flight experiments in 1-g. These can be performed repetitively prior to flight to test all equipment and experimental conditions (timing of sampling, density of culture).

7. Hardware Requirements**A. Minimum on Orbit Requirements**

CO₂ cell incubator, 1-g CO₂ cell incubator, sterile sampling/set up environment (laminar flow hood), fluorescence microscope with water and oil immersion lenses, filters, video camera, and data storage device.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, work area, fluid handling tools, compound microscope, data storage.

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Bernd Fritzschn

Department of Biomedical Sciences
Division of Anatomy
Creighton University
Omaha, Nebraska 68178

Critical Question(s) Addressed:

DB-2, DE-2, DG-1, DC-4 (See p. A-6)

3. Experiment Title

Specificity of Reconnecting of Gravity-sensing Organs in Microgravity

4. Purpose/Hypothesis

Reconnecting of an ear to the brain is achieved rapidly and is apparently specific. This repair could be specific based on cellular recognition alone or could be driven by the activity elicited through gravity on the gravity receptors. This experiment will test whether gravity sensing organs of the frog ear will specifically reconnect in the absence of the relevant stimulus, gravity.

5. Scientific Rationale/Rationale for Microgravity

Two receptor organs of the ear, the utricle and to some extent the saccule, are gravitation receptors. If the ear is removed experimentally early in development of the clawed frog, *Xenopus laevis*, and reimplanted in the same or a rotated position, these organs are reconnected to the brain and an apparently normal function is regained within one week. The central projection of these reconnected organs will be compared to similarly treated controls reared under 1-g. The data in conjunction with swimming analysis will allow assessment of the influence of 0-g on the re-establishment of proper connections of a vertebrate gravity sensor.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Ears of developing *Xenopus laevis* (stage 27) will be removed with fine needles and reimplanted with 90° rotation or without any rotation. About 24-48 hours after healing of the ears are completed, the animals will be launched together with unrelated controls (a total of six batches). Three batches will be fixed while in orbit, the other three batches after landing. Connections to the brain will be assessed employing the diffusion of a lipophilic fluorescent dye along the nerve fibers of specific epithelia of the inner ear.

C. Number and Type of Specimen:

60 larval clawed frogs, *Xenopus laevis*. Weight, including water to maintain the animals, about 1500 grams.

D. Measurements/Sample Handling:

Preflight: Operate 40 animals, rotate and reimplant the ear in 20 animals, reimplant ears without rotation in 20 animals.

In-flight: Maintain animals in appropriate aquatic animal facility. Fixation of half the specimens while in orbit.

Postflight: Application of the fluorescent dye to selected epithelia, section of the specimen after diffusion of the dye, and data analysis employing 3D-reconstruction of the distribution patterns to compare 0-g with 1-g animals. (Use of a fluorescence microscope, a vibratome, and a computer is required.)

E. Specific Sample Analysis:

In-flight: Observe behavior of larvae: swimming and orientation towards light.

Postflight: Label specific connections, section and analyze the projection pattern of specific gravity receptor organs.

F. Experiment Controls:

In-flight: Animals without ear operation or without rotation.

Ground: Delayed parallel experiments of animals operated at similar stages.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Aquarium system with filters to be able to feed the larvae. Six separate compartments, each approximately 100 ml. Three fixative compartments (20 ml) attached. Video camera to record the movements.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (fresh water), data storage, chemical fixation capability, ambient storage.

Experiment Identification Code: DB-g D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Charles Dinsmore

Department of Anatomy
Rush Medical College
1653 West Congress Parkway
Chicago, IL 60612

Critical Question(s) Addressed:

DB-2, DG-3 (See p. A-6)

3. Experiment Title

Role of Gravity in Wound Healing, Tissue Repair and Animal Limb Regeneration

4. Purpose/Hypothesis

Microgravity has a demonstrated effect on the deposition of collagen and synthesis of various hormones. Therefore, the normal processes of wound healing, tissue repair and regeneration may be effected. There also may be developmental mechanisms to compensate for the microgravity situation which could be studied in a space laboratory environment.

5. Scientific Rationale/Rationale for Microgravity

Many of the factors which might be expected to effect wound healing, tissue repair or limb regeneration are known to be influenced by lack of gravity. Most notably, the deposition of collagen, calcification and hormone responses have been shown to be effected by microgravity conditions. Therefore, it would be of interest to study these phenomena in a space laboratory environment.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 or 3

B. General Approach Description:

Generate wounds or amputate limbs of amphibians on board the space craft. The healing or regeneration processes will be studied in two phases. First, the wounds or blastema can be observed during the process of regeneration or repair by time lapse video. Second, the tissues can be fixed and analyzed at laboratories on Earth.

C. Number and Type of Specimen:

Many types of specimen would be of value for analysis.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Generate wounds or amputate limbs of amphibians. Time-lapse video of regeneration and repair. Tissue fixation.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of tissues.

F. Experiment Controls:

In-flight: Equivalent samples and experiments should be incubated in a space-based centrifuge at 1-g and processed as above.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Habitat environments for the different animals. Microscopy and video equipment. Glovebox and containment for fixing tissues.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (fresh water / salt water), [temperature controlled chamber], data storage, fluid handling tools, compound microscope, work area, dissection equipment, chemical fixation capability, ambient storage.

Experiment Identification Code: DB-h

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Patricia Y. Hester

Department of Animal Sciences
Purdue University
West Lafayette, IN 47907

Critical Question(s) Addressed:

DA-2, DB-1, DB-2, DG-1 (See p. A-6)

3. Experiment Title

Microgravity Effects on Avian Embryogenesis

4. Purpose/Hypothesis

To determine if microgravity affects avian embryonic movement, formation and location of major body organs, changes cell fluid movements, assembly of microtubules and microfilaments, mobility and intercellular communication during histogenesis and organogenesis, deposition of bone, formation of valve/vein/lymph vessels, migration of gonadocyte to the genital ridge, and metabolism and mobilization of minerals (calcium) from the shell.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have shown that 2-day-old chick embryos incubated in space were unable to develop normally and died prematurely. However, the 9-day-old embryos developed normally in 5 days of microgravity. The chicks which were allowed to hatch in the 9-day-old embryo group grew to be normal chickens which reproduced a second generation of chicks.

The cause of death of the 2-day-old embryos is unknown. Our hypothesis includes microgravity, cosmic radiation, not turning the eggs, or a combination of these factors as contributing to the death of the 2-day-old embryos.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 or 2 (1-16 or 9-13 days)

B. General Approach Description:

Avian embryos (quail or chicken) will be incubated in both 0-g and 1-g conditions for duration of flight (9 to 16 days). Two ages of avian development will be launched (e.g. 2- and 9-day-old embryos) depending on duration of flight. Half of the embryos will be opened after landing for examination and tissue processing. The remaining half will be allowed to develop for post-hatch performance and life cycle studies.

C. Number and Type of Specimen:

64 fertilized chicken eggs or 128 quail eggs for flight and an equal number for ground based controls.

D. Measurements/Sample Handling:

Preflight: Set selected eggs into incubator.

In-flight: Little or no crew interaction.

Postflight: Open 1/2 of the embryos and transfer the other half to hatcher.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Tissue and sample collection and fixation.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls with parallel in flight experiment in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

0-g and 1-g incubator for avian eggs with a turning device

B. GBF Hardware Capabilities Required (on Orbit)

Avian egg incubator, data storage, dissection equipment, fluid handling tools, chemical fixation capability, ambient storage.

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Richard Wassersug

Department of Anatomy & Neurobiology
Dalhousie University
Sir Charles Tupper Bldg.
Halifax, Nova Scotia
Canada B3H 4H7

Critical Question(s) Addressed:

DA-2, DB-1, **DB-2**, DB-3, DB-4, DF-2,
DG-1, DG-2, DG-3, DG-4 (See p. A-6)

3. Experiment Title

Microgravity Effects on Osteogenesis in Anurans

4. Purpose/Hypothesis

To determine: 1) whether exposure to microgravity affects primary osteogenesis in juvenile amphibians; and 2) if development in an aqueous environment naturally mitigates the detrimental effects of microgravity on the skeletal system. The hypothesis is that certain skeletal elements are more influenced by gravity than others, and as such, there should be observable differences in ossification in organisms that develop in microgravity, depending on whether they develop in water or not.

5. Scientific Rationale/Rationale for Microgravity

Most studies to date on the effects of microgravity on the vertebrate skeleton have examined the effects on the mature mammalian skeleton. Little, however, is known about how gravity affects initial skeletal development in general, and particularly in aquatic animals, which are normally near neutral buoyancy. The axial skeleton of aquatic animals may be similarly loaded, by reactive forces from the surrounding water, whether in microgravity or normal 1-g. This suggests that aquatic and terrestrial amphibians may exhibit different patterns of skeletal response to microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and (30 day Phase 2)

B. General Approach Description:

Amphibians of four species will be raised from larval stages to post-metamorphosis in microgravity and on a 1-g centrifuge on the Space Station. Two of the species (*Xenopus laevis* and *Ambystoma mexicanum*) have aquatic adults and two (*Rana pipiens* and *Ambystoma maculatum*) have terrestrial adults. The growth chambers will have air-spaces and structural supports to allow the animals to move out of the water at the time of metamorphosis. Specimens will be euthanized and fixed in space before return to Earth for histological study.

C. Number and Type of Specimen:

Mature, but premetamorphic larvae of the aquatic frog *Xenopus laevis*, the terrestrial frog *Rana pipiens*, the axolotl *Ambystoma mexicanum*, and the closely related mole salamander *Ambystoma maculatum*. Approximately 20 per larval chamber, with replicates.

D. Measurements/Sample Handling:

Preflight: Establish stocks and load chambers or transport vessels with specimens of comparable premetamorphic stage.

In-flight: Remove, euthanize and fix specimens.

Postflight: Clear and stain (with alcian blue and alizarin red) specimens to determine rate of cartilage and bone development in whole mounts of all specimens. (Requires good dissecting microscope, fiber optic illuminators, etc.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Detailed descriptive morphology of the skeletal system; possible calcium analysis of selected skeletal elements in specimens from all four species.

F. Experiment Controls:

In-flight: 1-g centrifuge controls.

Ground: Independent ground controls maintained [i.e. similar sample sizes, temperatures and containers] using individuals from the same clutches.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Growth chambers with and without 1-g centrifugation; facilities for removing aquatic specimens from growth chambers and triple-containment formalin fixation facilities.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (fresh water), data storage, fluid handling tools, work area, chemical fixation capability, ambient storage.

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s): Dr. Emily M. Holton

Mail Stop 236-7

NASA Ames Research Center

Moffett Field, CA 94035

(See Appendix H, Reference # 4: Experiment CH-D)

Critical Question(s) Addressed:

DB-1, DB-2, DB-3 (See p. A-6)

3. Experiment Title

Effects of Microgravity on Skeletal Growth, Maturity, and Calcium Metabolism

4. Purpose/Hypothesis

To determine bone changes which occur in rats during 1 year in flight.

Hypothesis: (a) rats, sent into space as weanlings, will show significantly less skeletal growth than their Earth-based counterparts; (b) the size, shape, and number of bones in flight animals will be different from ground-raised animals; (c) responses to provocative stimuli will be different in flight animals; and (d) readaption to 1-g will be more difficult with advancing age.

5. Scientific Rationale/Rationale for Microgravity

Gravity plays a major role in determining the size and shape of the skeletal system and is thought to be responsible for fusing many bones during growth. Rats achieve skeletal maturity within 1 year of life. This experiment will explore the importance of gravity on growth and development of the rat skeletal system from weanling to 1 year of age.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (1 year)

B. General Approach Description:

40 rats will be flown on the Space Station for up to one year. Body mass, bone markers, and response to calcium load/deprivation will be measured every 90 days on animals to be euthanized at one year. Blood will be drawn for analysis and bones will be removed from 5 rats every 90 days. Five rats will be returned to 1-g every 90 days. Electron microscopy, histomorphometry, and biochemical analysis will be performed postflight. Control experiments will be performed in-flight on a 1-g centrifuge and on the ground.

C. Number and Type of Specimen:

40 rats, 17-21 days old (rats born in-flight would be preferable if available; both F1 and F2 generations could be used).

D. Measurements/Sample Handling:

Preflight: X-ray of total skeleton, body mass (if born in-flight, these measurements would be made in-flight).

In-flight: Body mass, bone markers, response to calcium load/deprivation every 90 days only on rats to be euthanized at 1 year. Draw blood for analysis, remove bones from 5 rats every 90 days; collect urine/feces pools weekly and preserve for analysis.

Postflight: Similar to -in-flight.

E. Specific Sample Analysis:

In-flight: Separate serum from blood and freeze.

Postflight: Perform electron microscopy, histomorphometry, or biochemical analysis on bones. Analyze urine/feces and blood components.

F. Experiment Controls:

In-flight: 1-g centrifuge controls; see flight animals for details.

Ground: 20 rats, 17-21 days old.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Holding facility that will support rats from weanlings through adulthood (0.08-0.6 kg), rat guillotine, refrigerated centrifuge, fixation kit, -70°C freezer, small mass measure, glovebox, nonradioactive tracer kit, surgical supplies, 1-g on-board centrifuge. X-ray machine (if animals born in-flight).

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, rodent birthing habitat, rodent rearing habitat (for rats born in-flight), data storage, dissection equipment, temperature controlled laboratory centrifuge, fluid handling tools, chemical fixation capability, work area, mass measurement device, -70°C freezer, ambient storage, refrigeration (4°C).

Experiment Identification Code: DB-k D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s): Dr. Emily M. Holton

Mail Stop 236-7

NASA Ames Research Center

Moffett Field, CA 94035

(See Appendix H, Reference # 4: Experiment CH-G)

Critical Question(s) Addressed:

DB-2, DB-3 (See p. A-6)

3. Experiment Title

Relationship Between Bone Formation and Bone Resorption Defects in Microgravity

4. Purpose/Hypothesis

Purpose: to determine whether (and how) the normally-coupled processes of bone formation and bone resorption are uncoupled in microgravity.

Hypothesis: the known defect in osteoblast function in flight may modify the bone-resorptive response in both the juvenile and adult skeletons.

5. Scientific Rationale/Rationale for Microgravity

Bone loss in the adult skeleton can be due to decreased formation or increased resorption or both. The known defect in osteoblast maturation or function may be accompanied by decreases in skeletal coupling factors. It is necessary to know this if appropriate countermeasures are to be developed for long-term flight; the first piece of information necessary is the relationship between formation in both modeling and remodeling skeletons.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (120 days)

B. General Approach Description:

Bone resorption and formation in juvenile and adult rats exposed to spaceflight will be analyzed using electron microscopy, histomorphometry, biochemical analysis and stable calcium isotope tracer studies.

C. Number and Type of Specimen:

40 rats (10 each at 30, 60, 90, and 120 days of age).

D. Measurements/Sample Handling:

Preflight: Tracer injections, animal sacrifice, specimen fixation, stable calcium isotope tracer studies.

In-flight: Same as preflight.

Postflight: Same as preflight.

E. Specific Sample Analysis:

In-flight: Perform electron microscopy, histomorphometry, or biochemical analysis on bone.

Postflight: Same as in-flight.

F. Experiment Controls:

In-flight: 1-g centrifuge controls; see flight animals for details.

Ground: 40 rats; see flight animals for details.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Holding facility for rats, rat guillotine, refrigerated centrifuge, surgical supplies, -70°C freezer, glovebox, nonradioactive tracer kit, refrigerator (4°C), sonomicrometer, electron microscope, specimen fixation equipment, 1-g on-board centrifuge.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage, -70°C freezer, fluid handling tools, dissection equipment, chemical fixation capability, work area, refrigeration (4°C), ambient storage.

Experiment Identification Code: DB-1

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-S)

Critical Question(s) Addressed:

DB-2, DB-3 (See p. A-6)

3. Experiment Title

Muscle Maturation of Insects in Space

4. Purpose/Hypothesis

Maturation of the insect flight muscle may be altered in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Uncoordinated and altered flying activity of insects raised in microgravity is expected to inhibit the normal 1-g maturation of the insect flight muscle.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Insects will be collected at different ages from the initial population, homogenized, and prepared for postflight enzyme analysis.

C. Number and Type of Specimen:

100 male fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Collect insects at different ages from the initial population, homogenize, and prepare for enzyme analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Determine activity of marker enzyme, e.g., cytochrome oxidase.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Controls as per flight insects.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Fluid handling kit, spectrophotometer, homogenizer, insect module, glovebox, refrigerator (4°C).

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage, fluid handling tools, refrigeration (4°C), -70°C freezer, work area, spectrophotometer.

Experiment Identification Code: DB-m D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Olaf Ellers

Department of Zoology
University of California, Davis
Davis, CA 95616

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

Biological Material Property Changes in Microgravity

4. Purpose/Hypothesis

To measure material property changes due to microgravity in the sea urchin skeleton.

5. Scientific Rationale/Rationale for Microgravity

The mechanical properties of biological materials can be expected to change when these materials go into a non-gravitational environment in space. Mechanical tests could be done on the material in space. One test that is particularly revealing is vibration testing. Because vibration depends on several properties of the material, vibrational testing could be used to detect early and rapid changes in the material composition of the material.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Changes in skeletal composition and structure can be detected by striking the skeleton with a small mallet and recording the sound that results (the "pinging test"). The pattern of sound indicates the degree of change that has taken place. A more sophisticated version of the "pinging test" is to vibrate the skeleton and measure which frequencies the material transmits.

Adult sea urchins would be carried into orbit. Then, every hour, one urchin would be sacrificed and the vibrational test would be performed.

C. Number and Type of Specimen:

Sea urchins.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Sacrifice sea urchins once per hour and perform vibrational testing.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Vibrational analysis.

F. Experiment Controls:

In-flight: None.

Ground: Ground-based controls

7. Hardware Requirements

A. Minimum on Orbit Requirements

Microphones and a spectrum analyzer for the "pinging test". A vibrator, strain gages and a two-channel spectrum analyzer for the more sophisticated vibration test.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (salt water), [temperature controlled chamber], data storage, work area, dissection equipment, chemical fixation capability.

Experiment Identification Code: DB-n D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Olaf Ellers

Department of Zoology
University of California, Davis
Davis, CA 95616

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

The Role of Gravity in the Force Balances Governing Growth, Calcification and Shape in Sea Urchins

4. Purpose/Hypothesis

I model sea urchin shapes by calculating the shape that eliminates bending stresses in the shell when it is loaded by gravity and a fluctuating internal pressure that I have measured in sea urchins. This model correctly predicts the shape of sea urchins on Earth. A powerful test of the theory would be to grow the urchins in microgravity since predicted shapes would be very different.

5. Scientific Rationale/Rationale for Microgravity

Mathematical modeling indicates that sea urchin shape and calcification is determined by both a pressure gradient created by gravity acting over the height of the urchin as well as an internal coelomic pressure generated by the urchin using its peristomial membrane. The relative size of this internal pressure and the gravitational pressure gradient determine the force balance at each point in the sea urchin's skeleton. If bending stresses are eliminated, that force balance specifies a unique shape. Alterations in final shape and pattern of calcification that occur in the absence of gravity will test the mathematical model.

Similar mathematical methods could be used to predict the shape of cells under specified internal pressures and external pressure gradients. Shape changes in cells could be responsible for a large number of bizarre consequences in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 or 2 (16 or 30-180 days)

B. General Approach Description:

Phase 1: Sea urchin larvae will be induced to settle in space. Early post-larval growth occurs over a few days and involves the formation of the first skeletal plates. The larvae will be preserved in space and the shapes will be measured on Earth. Alternatively, the developing shape could be filmed in space, and the film analyzed. Control groups would be grown in space in the centrifuge.

Phase 2: Adult sea urchins would be launched into space, grown there, and brought back after 3 months. Experiments on Earth suggest that the material properties of calcite can change with diet manipulations in three months. In space, both calcification and shape should change over that period of time.

C. Number and Type of Specimen:

Strongylocentrotus purpuratus. 6 groups (3 control and 3 experimental) of 10 sea urchins each *Strongylocentrotus purpuratus* (total = 60 urchins).

D. Measurements/Sample Handling:

Preflight: Grow several extra sets larvae so that one group will be at the right stage in development at launch.

In-flight: Let the juvenile urchins grow in sea water. Provide food. Provide appropriate filtering and replacement of water. Possibly film them growing under a microscope. Add tetracycline to label calcite growth. Finally, add preservative.

Postflight: Analyze shapes. Measure brittleness and density of the calcite. View calcite plates using UV light to compare calcification patterns. Measure elemental and organic composition of the plates and observe plate shape details using electron microscopy.

E. Specific Sample Analysis:

In-flight: Filming.

Postflight: Electron microscopy, density, elemental analysis, histology, mechanical testing (vibrational and bending tests to determine material property changes), mathematical and statistical analysis.

F. Experiment Controls:

In-flight: 1-g controls (actually controls or experimental treatments at other g's would be useful too, because the mathematical model could be tested at several g levels.)

Ground: Controls to parallel in-flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

- 1) Sea water aquaria with filters for excreted wastes and inputs of food (algae, fresh or rehydrated).
- 2) A method for adding preservative to the sea water.
- 3) A method for adding tetracycline to the water.
- 4) A method for adding adult extract (to induce the larvae to settle).

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), medium aquatic habitat (salt water), data storage, compound microscope, fluid handling tools, work area, chemical fixation capability, refrigeration (4°C), ambient storage.

Experiment Identification Code: DB-o D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Wallace D. Berry

Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

Development of Avian Vitamin D Endocrine System in Microgravity: Vitamin D Metabolites and Mineral Homeostatic Hormones

4. Purpose/Hypothesis

Microgravity will cause changes in relative levels of vitamin D metabolites, parathyroid hormone, and calcitonin.

5. Scientific Rationale/Rationale for Microgravity

Adaptation to microgravity drives changes in bone physiology that result in bone mineral loss/redistribution and negative calcium balance. Corresponding changes should occur in mineral homeostatic hormones. These hormones are important in the function of a wide range of tissues. Therefore, the information gained in this study will be valuable in determining the mechanisms of microgravity effects and in anticipating long term effects.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Fertile chicken eggs will be incubated in-flight. Plasma or serum will be collected from embryos or chicks postflight and frozen (-70° or -196°C). Vitamin D metabolites, parathyroid hormone, and calcitonin will be assessed postflight.

C. Number and Type of Specimen:

Four samples drawn from a minimum of four flight eggs (16 samples). Additional samples if 1-g centrifuge is available in Avian Research Module.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: None.

Postflight: Blood draws, blood processing, sample freezing. (Blood draw equipment and sample vials, centrifuge for processing plasma or serum, and freezer (-70° or -196°C) required.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay of vitamin D metabolites, PTH, CT.

F. Experiment Controls:

In-flight: 1-g centrifuge controls if centrifuge available, otherwise none.

Ground: Controls will parallel flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Avian Research Module.

B. GBF Hardware Capabilities Required (on Orbit)

Avian egg incubator, data storage, refrigeration (4°C).

Experiment Identification Code: DB-p D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Wallace D. Berry

Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

Effects of Microgravity on Avian Vitamin D Receptors and Calcium Binding Proteins

4. Purpose/Hypothesis

Autoinduction and regulation of the vitamin D receptor, and expression of D dependent calcium binding proteins are affected by microgravity.

5. Scientific Rationale/Rationale for Microgravity

Vitamin D receptors and D dependent calcium binding proteins are found in most tissues or organ systems affected by microgravity including intestine, kidney, bone, brain, cardiac and smooth muscle, the vestibular system, and immune cells. Space flight and ground based models of microgravity affect mineral homeostasis and the vitamin D endocrine system. Tissue levels of vitamin D receptor and calcium binding proteins may be decreased as a result.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Appropriate tissues will be collected immediately postflight and prepared for assay of dihydroxyvitamin D receptors and calcium binding proteins calbindin D28k and calbindin D9k. Tissues may also be fixed and subjected to immunohistochemistry for identification of calbindin containing cells.

C. Number and Type of Specimen:

One sample of serum or plasma, duodenal loop, kidney, brain, and pancreas from each of four chicken embryos incubated in-flight. Identical samples from flight 1-g centrifuge if available.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: None.

Postflight: Blood draws and processing, sample dissection, storage of samples by fixation or freezing. (Dissection equipment, vials and fixatives, and freezer (-70° or -196°C) required.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay of vitamin D receptors, calbindins, calbinding immunohistochemistry

F. Experiment Controls:

In-flight: 1-g centrifuge controls if available, otherwise none.

Ground: Controls will parallel flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Avian Research Module.

B. GBF Hardware Capabilities Required (on Orbit)

Avian egg incubator, data storage, refrigeration (4°C).

Experiment Identification Code: DB-q

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Wallace D. Berry

Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

Vitamin D Endocrinology in Microgravity: Vitamin D Metabolites and Mineral Homeostatic Hormones

4. Purpose/Hypothesis

Microgravity will cause changes in relative levels of vitamin D metabolites, parathyroid hormone, and calcitonin.

5. Scientific Rationale/Rationale for Microgravity

Adaptation to microgravity drives changes in bone physiology that result in bone mineral loss/redistribution and negative calcium balance. Corresponding changes should occur in mineral homeostatic hormones. These hormones are important in the function of a wide range of tissues. Therefore, the information gained in this study will be valuable in determining the mechanisms of microgravity effects and in anticipating long term effects.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Plasma or serum will be collected from rats in-flight at regular intervals (2-4 days) and frozen (-70°C or -196°C). Vitamin D metabolites, parathyroid hormone, and calcitonin will be assessed postflight.

C. Number and Type of Specimen:

Four samples drawn from a minimum of four flight rats (16 samples). Additional samples if 1-g animal centrifuge is available.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Blood draws, blood processing, sample freezing.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay of vitamin D metabolites, PTH, CT

F. Experiment Controls:

In-flight: 1-g centrifuge controls if centrifuge available, otherwise none.

Ground: Controls will parallel flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Animal maintenance facilities (RAHF or AEM type are adequate), blood draw equipment and sample vials, centrifuge for processing plasma or serum, freezer (-70° or -196°C).

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage, fluid handling tools, temperature controlled laboratory centrifuge, -70°C freezer, work area.

Experiment Identification Code: DB-r

D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Wallace D. Berry

Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

Critical Question(s) Addressed:

DB-3 (See p. A-6)

3. Experiment Title

Effects of Microgravity on Vitamin D Receptors and Calcium Binding Proteins

4. Purpose/Hypothesis

Autoinduction and regulation of the vitamin D receptor, and expression of D dependent calcium binding proteins are affected by microgravity.

5. Scientific Rationale/Rationale for Microgravity

Vitamin D receptors and D dependent calcium binding proteins are found in most tissues or organ systems affected by microgravity including intestine, kidney, bone, brain, cardiac and smooth muscle, the vestibular system, and immune cells. Space flight and ground based models of microgravity affect mineral homeostasis and the vitamin D endocrine system. Tissue levels of vitamin D receptor and calcium binding proteins may be decreased as a result.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Appropriate tissues will be collected immediately postflight and prepared for assay of dihydroxyvitamin D receptors and calcium binding proteins calbindin D28k and calbindin D9k. Tissues may also be fixed and subjected to immunohistochemistry for identification of calbindin containing cells.

C. Number and Type of Specimen:

One sample of serum or plasma, duodenal loop, kidney, brain, and pancreas from each of four flight rats. Identical samples from flight 1-g centrifuge rats if available.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: None.

Postflight: Blood draws and processing, sample dissection, storage of samples by fixation or freezing. (Dissection equipment, vials and fixatives, freezer (-70° or -196°C) required.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay of vitamin D receptors, calbindins.

F. Experiment Controls:

In-flight: 1-g centrifuge controls if available, otherwise none.

Ground: Controls will parallel flight experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Animal maintenance facilities (RAHF or AEM type adequate).

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage.

Experiment Identification Code: DB-s D = developmental; B = later developmental events

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-V)

Critical Question(s) Addressed:

DA-7, DB-3, DB-4 (See p. A-6)

3. Experiment Title

Heat Shock Proteins in Insects in Space

4. Purpose/Hypothesis

Spaceflight stresses may induce the formation of "heat-shock" proteins or possibly of a new set of previously undescribed "stress-related" polypeptides.

5. Scientific Rationale/Rationale for Microgravity

It is well documented that *Drosophila* respond to a number of environmental stresses by synthesizing a class of polypeptides known as heat-shock proteins. It is expected that spaceflight stress, and in particular microgravity, may induce the expression of these or similar proteins.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Label insects with radioisotopes (¹⁴C-leucine, or ³⁵S-methionine) on-orbit . Homogenize insects in lysis buffer and store for postflight analysis.

C. Number and Type of Specimen:

25 male fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Label insects with radioisotopes (^{14}C -leucine, or ^{35}S -methionine). Homogenize insects in lysis buffer and store for postflight analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Two-dimensional gel electrophoresis and autoradiography.

F. Experiment Controls:

In-flight: None.

Ground: Controls as per in flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Homogenizer, insect habitat, glovebox, refrigerator (4°C).

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, [temperature controlled chamber], data storage, fluid handling tools, -70°C freezer, work area, radioisotope handling equipment.

Experiment Identification Code: DC-a D = developmental; C = parturition/postnatal growth

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s): Dr. Emily M. Holton

Mail Stop 236-7

NASA Ames Research Center

Moffett Field, CA 94035

(See Appendix H, Reference # 4: Experiment CH-E)

Critical Question(s) Addressed:

DB-1, DB-3, DC-1 (See p. A-6)

3. Experiment Title

Bone Response to Pregnancy and Lactation in Microgravity

4. Purpose/Hypothesis

To determine whether the skeletal response to pregnancy/lactation is altered by microgravity.

Hypothesis: The normal homeostatic responses of the skeleton and intestine during pregnancy/lactation will be blunted by microgravity because the bone cells will be less responsive.

5. Scientific Rationale/Rationale for Microgravity

Pregnancy/lactation is a well-characterized perturbation to calcium homeostasis which involves multiple endocrine systems. Microgravity can alter the response of this system in several ways, including both calcium endocrinology and pituitary function. The effect of microgravity on the interaction between calcium homeostasis and pituitary function is unknown.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Rats will be bred in-flight (except for the first group) at 0, 29, 59, 89, 119 days of flight. Pregnant rats will be euthanized at 20, 50, 80, 110, 140 days (at delivery). Lactating rats will be euthanized at 41, 71, 91, 131 and 161 days (at weaning). Quantitative histomorphometry, autoradiography of bone cells, and analysis of serum for gonadotropins and calciotropic hormones will be performed postflight.

C. Number and Type of Specimen:

50 breeding rats (bred in-flight except for first group). Each rat will be bred only once.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Breed 10 rats each at 0, 29, 59, 89, and 119 days of flight. Euthanize 5 pregnant rats each at specified intervals. Euthanize 5 lactating rats each at specified intervals. Tissue harvest after tracer and tetracycline injections, and serum analysis.

Postflight: Similar to in-flight.

E. Specific Sample Analysis:

In-flight: Serum analysis.

Postflight: Quantitative histomorphometry, autoradiography of bone cells, and analysis of serum for gonadotropins and calcitropic hormones will be performed postflight.

F. Experiment Controls:

In-flight: 1-g centrifuge controls; see flight animals for details.

Ground: 50 breeding rats; see flight animals for details.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Rodent development modules, laboratory centrifuge, refrigerated centrifuge, fixation kit, -70°C freezer, guillotine, glovebox, nonradioactive tracer kit, rodent module, tissue harvest equipment, on-board 1-g centrifuge with breeding facilities.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent breeding habitat, rodent rearing habitat, rodent weanling to adult habitat, data storage, dissection equipment, temperature controlled laboratory centrifuge, fluid handling tools, work area, -70°C freezer, chemical fixation capability, refrigeration (4°C), ambient storage.

Experiment Identification Code: DD-a D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Carey R. Phillips

2. Author(s): Dr. Carey R. Phillips

Department of Biology
Bowdoin College
Brunswick, ME 04011

Critical Question(s) Addressed:

DA-2, DB-1, DD-1, DG-3 (See p. A-6)

3. Experiment Title

Study of the Evolutionary Pressures Exerted by Earth's Gravitational System

4. Purpose/Hypothesis

There are certain physical constraints and developmental parameters normally exerted by Earth's gravitational system which would be altered in a microgravity environment. A long term culture of several species would begin to define these constraints and parameters.

5. Scientific Rationale/Rationale for Microgravity

Several species of embryos will orient themselves relative to Earth's gravity during some phase of their development. It has also been demonstrated that altered gravitational environments can have a marked effect, in some species, on embryonic development. In addition, it has been demonstrated that cells and some embryos subjected to different pressures will alter their normal developmental pattern. Therefore, it is of interest to study the long term effects of microgravity on the developmental profile of several species in order to understand the selection pressures exerted by Earth's gravitational environment on development at both the embryo and the cellular level.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 - 90 days or longer

B. General Approach Description:

Grow multiple generations of several different fast generating species and analyze the embryos and cellular pattern of development.

C. Number and Type of Specimen:

These experiments will require a large sample size and would be best served if several species could be studied. Pond snails and fruit flies offer two very good opportunities to study very different types of development, while offering relatively fast generation times.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Embryos from several developmental stages of each generation would be fixed for ground based analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Studies on rates of development, effects of microgravity on numbers of progeny produced and mating behavior of the individuals.

Postflight: Analyze embryos and cellular pattern of development using standard molecular techniques.

F. Experiment Controls:

In-flight: Embryos would be grown in a centrifuge facility under simulated gravity of 0.5, 1.0, and 2.0 times gravity.

Ground: Ground based studies would be conducted with the same genetic strain.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Growth chambers for the organisms. Inflight mmicroscopes for initial observations and ability to fix the different stages of embryos.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, data storage, medium aquatic habitat (fresh water), compound microscope, chemical fixation capability, work area, ambient storage.

Experiment Identification Code: DD-b D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-P)

Critical Question(s) Addressed:

DD-1, DE-2 (See p. A-7)

3. Experiment Title

Multigeneration Reproduction in Insects

4. Purpose/Hypothesis

The purpose of this experiment is to determine whether multiple generations can successfully be obtained in microgravity and to compare late-generation insects with late-generation ground controls.

5. Scientific Rationale/Rationale for Microgravity

Need to determine whether multigenerations can be obtained in microgravity and whether animals produced under these conditions experience selection pressures (e.g., male, female) and whether late generation organisms are normal compared to ground-based insects.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Insects will be allowed to reproduce through multiple generations for at least 90 days (8 generations). At the end of the flight, the "module" containing the last generation of insects and their prospective offspring will be sent to Earth for analysis. After return, body weights, sex ratios, percent of eggs that develop, lifespan, and other physiological and biochemical parameters will be examined to determine "normalcy".

C. Number and Type of Specimen:

Start with 20 male and 30 female fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Insects will be allowed to reproduce through multiple generations for at least 90 days (8 generations). At the end of the flight, the "module" containing the last generation of insects and their prospective offspring will be sent to Earth for analysis.

Postflight: Examine body weights, sex ratios, percent of eggs that develop, lifespan, and other physiological and biochemical parameters designed to determine "normalcy" of the insects returned for analysis. (Balance required for measuring body weights.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Same as in-flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Special platform module (pressurized, temperature regulation, light, etc.) for insects.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, data storage.

Experiment Identification Code: DD-c D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-I)

Critical Question(s) Addressed:

DA-2, DA-6, DD-1 (See p. A-6)

3. Experiment Title

Multigenerational Production of Avian Species

4. Purpose/Hypothesis

Determine the capability of avian species to carry out a complete life cycle in microgravity, and determine the establishment of correct anteroposterior embryo axis.

5. Scientific Rationale/Rationale for Microgravity

Rotation of avian egg during early embryogenesis is potentially gravity-sensitive. Study nonplacental development and life cycles in avian model.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Quail will be artificially inseminated in flight. Fertile eggs will be collected and stored at 4°C for 1 week. Eggs will be incubated at 37°C, 65% RH to hatch (~17d). Sacrifice schedule of embryos and developmental morphology are TBD. Hatchlings will be harvested, segregated by sex and housed in "hatchling facility", and sampled TBD.

C. Number and Type of Specimen:

8 female and 3 male quail, *Coturnix japonica*.

D. Measurements/Sample Handling:

Preflight: Install birds in avian holding facility for 2-wk adaptation.

In-flight: Wk 1: artificially inseminate; collect and store fertile eggs (4°C). Wk 2: incubate eggs. Sacrifice schedule and developmental morphology are TBD. Harvest and segregate hatchlings. Retain eight females for F2 generation. Return samples to Earth.

Postflight: Wk 1: artificially inseminate eight F1 females to test viability of ova formed in-flight.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of samples collected in-flight.

F. Experiment Controls:

In-flight: 1-g centrifuge controls.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Avian habitat, incubator, fixation kit, freezer (-20°C), variable gravity centrifuge (for controls), guillotine, glovebox, refrigerator (4°C), dissection equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Avian adult habitat, avian egg incubator, avian hatchling habitat, data storage, chemical fixation capability, work area, dissection equipment, refrigeration (4°C).

Experiment Identification Code: DD-d D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference #4: Experiment R/D-A)

Critical Question(s) Addressed:

DD-1, DE-2 (See p. A-7)

3. Experiment Title

Multiple-Generation Studies of Mammals in Space

4. Purpose/Hypothesis

To determine the feasibility of producing successive generations of rodents in space.

5. Scientific Rationale/Rationale for Microgravity

The mammalian life cycle (reproductive and development) evolved in the presence of gravity. Can the mammalian life cycle be expressed in microgravity? Ultimately, space habitation will require unimpaired reproduction and development. Human safety after space exposure is an ethical necessity. Science and commercial interests will require vivaria and space-adapted animals. Basic biological questions can be posed and answered.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Animal mass, food and water consumption will be measured in flight. Continuous video data will be recorded. Data analysis will be performed postflight.

C. Number and Type of Specimen:

10 female, 4 male each rats and mice.

D. Measurements/Sample Handling:

Preflight: Reproductive rates and fecundity (proven breeders).

In-flight: Weights at intervals, continuous video, and food and water intake. Cull litters soon after birth, and fix culls.

Postflight: Weights, behavior, allometry, readaptation, and breeding.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of data collected in-flight.

F. Experiment Controls:

In-flight: None. Centrifuge controls are not essential at this stage.

Ground: Littermate vivarium and synchronous controls. (The sibling matings on Earth-siblings of space rodents-are the critical controls).

7. Hardware Requirements

A. Minimum on Orbit Requirements

Rodent breeding/rearing habitat, -20°C freezer, guillotine, mass measure (small), glovebox, fixation capability, video camera/VCR, feeding monitor.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent rearing habitat, rodent breeding habitat, data storage, fluid handling tools, mass measurement device, work area, dissection equipment, chemical fixation capability, ambient storage.

Experiment Identification Code: DD-e

D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Wallace D. Berry

Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

Critical Question(s) Addressed:

DD-1 (See p. A-7)

3. Experiment Title

Ontogeny of Vitamin D Dependent Systems in Microgravity

4. Purpose/Hypothesis

Gestation and postnatal development will alter development of vitamin D dependent mineral homeostatic systems.

5. Scientific Rationale/Rationale for Microgravity

Vitamin D dependent calcium absorption and homeostasis is not fully developed in the rat until approximately postnatal day 21. A critical period for induction of vitamin D receptors and dependent calcium binding proteins may occur during this time. Therefore, microgravity effects on calcium metabolism in the gestating female may influence the development of components of the vitamin D endocrine system in offspring.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 37 days

B. General Approach Description:

Pregnant rats maintained on orbit from gestation day 5 or earlier through birth of pups and weaning. Blood and appropriate tissues will be collected in-flight. Plasma or serum and tissue samples will be frozen or fixed as appropriate for transfer to ground facilities. Vitamin D metabolites, PTH, CT, dihydroxyvitamin D receptors, and calbindins will be analyzed.

C. Number and Type of Specimen:

One sample of serum or plasma, intestine, kidney, brain, and pancreas will be taken from each pup (assume 8-10 pups/dam) and dam. Identical samples taken from flight 1-g centrifuge rats if possible.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Mass measurement of dams and pups. Blood draws and processing, sample dissections, storage of samples by fixation or freezing.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Assay of vitamin D metabolites, PTH, CT, vitamin D receptors, calbindins. Immunohistochemistry of calbindins.

F. Experiment Controls:

In-flight: 1-g centrifuge controls if available.

Ground: Controls will parallel flight experiments.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Animal maintenance facilities (RAHF or IAEM type are adequate). Blood draw and dissection facilities (GPWS type adequate). Centrifuge for blood processing. Vials and freezer for sample storage (-70°C or -196°C).

B. GBF Hardware Capabilities Required (on Orbit)

Rodent birthing habitat, rodent rearing habitat, data storage, work area, fluid handling tools, temperature controlled laboratory centrifuge, -70°C freezer, chemical fixation capability, mass measurement device, dissection equipment, ambient storage.

Experiment Identification Code: DD-f D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Lynn Wiley

Department of OB/GYN
University of California School of Medicine
Davis, CA 95616

Critical Question(s) Addressed:

DD-1, DE-2 (See p. A-7)

3. Experiment Title

Serial Generations of Mice in Space

4. Purpose/Hypothesis

The purpose of this experiment is to make initial observations and answer basic questions regarding the successes or failures of reproduction and development of mammals (mice) over serial generations in space.

5. Scientific Rationale/Rationale for Microgravity

NASA has been given the mandate to enable humans to inhabit space for long periods of time (months and years). Consequently it has now become absolutely essential that we learn all we can about the effects that space will have on the reproductive status of humans, whether they bear children upon return to Earth, while in residence on extraterrestrial bodies, or in free space. To date, no animal species has successfully gone 'egg-to-egg' in space, nor has any vertebrate embryo successfully developed to adulthood in space. If we want to learn about human reproduction and development in space, experiments are needed using appropriate animal models.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Breeding pairs of mice will be flown on the Space Station under appropriate habitat conditions and observed for their inclination and ability to breed. If litters result, these will be weaned and sexed at 3 weeks of age and separated by sex. When (if) they attain breeding age, they will be paired off and observed as were their parents. Provided that progeny are obtained and they develop to maturity, this process can be repeated until sufficient observations have been obtained.

Reproductive success will be assessed by: 1) number of pairs that successfully copulate, 2) number of females that become grossly pregnant, 3) time of gestation, 4) litter size 5) frequency of pregnancies 6) average reproductive life span, 7) average lifetime of parents. Developmental success will be assessed by: 1) average weight and crown-rump length of pups 2) sex ratio within a litter 3) average time to weaning 4) average number that survive to adulthood (and of those, (a) average size (b) sex ratio)

C. Number and Type of Specimen:

Five breeding pairs of 6-8 week old CD1 (ICR) outbred Swiss strain mice. The National Cancer Institute has an extensive data base on the incidence of a variety of tumors in this specific mouse strain. Mice will be used because they have a long history of serving as a model for studies on mammalian development and reproduction. Their small size (25-35 grams), short generation time (3 to 6 weeks), and large litters (10-20 liveborn young per litter) make them attractive candidate models.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Observations of reproductive and developmental endpoints as described above.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Parallel cohorts of ground controls will be appropriately housed and maintained for the duration of the experiment on the Space Station.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Breeding and rearing environment (vivarium) for mice, video monitoring capabilities.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent breeding habitat, rodent birthing habitat, rodent rearing habitat, work area, mass measurement device, data storage.

Experiment Identification Code: DD-g D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Lynn Wiley

Department of OB/GYN
University of California School of Medicine
Davis, CA 95616

Critical Question(s) Addressed:

DD-1, DE-2 (See p. A-7)

3. Experiment Title

Serial Generations of Mice in Space Follow-on: Reproductive Function of Space-Raised Mice After Return to Earth

4. Purpose/Hypothesis

If serial generations are shown to be a possibility for mammals in space (determined from the results of the previous experiment) and space has no apparent effect on mammalian reproduction over generations so long as the parents and progeny remain in space, this experiment will determine if mice born and matured in space exhibit normal reproductive function if returned to Earth.

5. Scientific Rationale/Rationale for Microgravity

The prospect of long term habitation in space for humans requires that we determine the effects that space will have on the reproductive status of humans while resident in space and after return to Earth. This experiment will test the reproductive success of mammals (mice) that were born and reared on the Space Station and the developmental success of their progeny.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (up to 8 months)

B. General Approach Description:

Mice that were conceived, gestated, born and matured on the Space Station will be tested (observed) for the following endpoints to assess reproductive and developmental success:

Reproduction: 1) number of breeding pairs that successfully copulate, 2) number of females that become grossly pregnant, 3) time of gestation, 4) litter size 5) frequency of pregnancies 6) average reproductive lifespan, 7) average lifetime of parents.

Development: 1) average weight and crown-rump length of newborn pups 2) sex ratio of pups within a litter 3) average time to weaning 4) average number of pups per litter that survive to adulthood (and of those, (a) average size (b) sex ratio)

C. Number and Type of Specimen:

CD1 (ICR) outbred Swiss strain mice that were born and reared on the Space Station in 0-g. (The number is dependent on the outcome of the previous experiment).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: None.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Observations of reproductive and developmental endpoints as described above.

F. Experiment Controls:

In-flight: None.

Ground: Parallel cohorts of ground controls will be appropriately housed and maintained for the duration of the experiment on the Space Station.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Breeding and rearing environment (vivarium) for mice, video monitoring capabilities.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent breeding habitat, rodent birthing habitat, rodent rearing habitat, work area, mass measurement device, data storage.

Experiment Identification Code: DD-h D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

Reference SWG Member

Bruce Hammock

2. Author(s): Harry Kaya

Department of Nematology
University of California
Davis, CA 95616

Critical Question(s) Addressed:

DD-1, DB-2

3. Experiment Title

A Comparison of Insect-parasitic Nematodes

4. Purpose/Hypothesis

To determine if the behavior and infectivity of *Steinernema carpocapsae* which is a nictating species and *Steinernema glaseri* which is an active searcher are affected by weightlessness. If infection occurs, can the nematodes develop normally? Do they mate and produce progeny? How does microgravity affect the nematodes' fitness and morphology?

5. Scientific Rationale/Rationale for Microgravity

To understand the role gravity plays in nictating behavior and subsequent cues required by the nematodes to infect host insects.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1; 8 days or less

B. General Approach Description:

Prepare petri dishes with moist filter paper or sterilized sand. Add nematodes and insects to the petri dishes, one group containing the nictating nematode species, and the other group containing the active searchers. Determine insect mortality for both groups. Remove dead insects to "white traps" and collect progeny from both nematode groups.

C. Number and Type of Specimen:

Nematodes: *Steinernema carpocapsae* and *Steinernema glaseri* ; closely related to *C. elegans* but confined to an insect contained environment, 20 nematodes per insect

Insects: *Galleria mellonella* or other suitable lepidopteron larvae; 10 insects per petri dish.

D. Measurements/Sample Handling:

Preflight: None

In-flight: Add insects and nematodes to petri dishes. Move dead insects within 48 hrs into clean chambers.

Postflight: Set up dead insects in "white traps" to collect progeny.

E. Specific Sample Analysis:

In-flight: None

Postflight: Count nematode progeny from dead insects in both groups. Perform microscopic examination of progeny. Study infectivity of progeny.

F. Experiment Controls:

In-flight: No nematodes added to petri dishes, only insects.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Petri dishes with both smooth and rough surfaces. Forceps useful for manipulation of specimens.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, work area, data storage.

Experiment Identification Code: DE-a D = developmental; E = behavior/circadian rhythms

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Marianne K. Steele

Dept. of Physiology
University of California
San Francisco, CA 94143

Critical Question(s) Addressed:

DE-1 (See p. A-7)

3. Experiment Title

Body Rhythms in Rodents

4. Purpose/Hypothesis

To determine the effects of microgravity on basic biological rhythms.

5. Scientific Rationale/Rationale for Microgravity

Physiological functions are characterized by rhythms, i.e. neuronal firing rates, endocrine hormones, reproductive behavior, temperature, sleep-wake cycles and eating patterns. Consistent and regular rhythms are indicative of normal physiological functioning. The proposed experiment will determine effects of microgravity and altered light/dark schedules on obvious, easily quantifiable rhythms. Alterations in these rhythms will provide clues to altered behavior, endocrine and neural (cognitive) function and provide a basis for more complex experiments.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 30 days

B. General Approach Description:

Rats will be maintained in individual metabolic cages. The cages will be instrumented such that daily feeding, drinking and activity levels (sleep-wake cycle) can be monitored, and feces and urine collected (for analysis of body fluid exchange and various hormones). The involvement of on-board crew will be essential in maintaining food and water supply, removal and storage of waste products and weekly weighing of the animals. Telemetry may be used for body temperature.

C. Number and Type of Specimen:

Start with 5 male and female rats. Female rats exhibit a 4 day estrous cycle, with activity, temperature and drinking rhythms closely tied to the adrenal and reproductive hormone rhythms. Aberrations in these behavioral indices will suggest aberrations in endocrine function.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Computer acquisition of temperature, feeding and activity data.

Postflight: Sacrifice of the animals, collection of blood and tissues, data analysis. (Dissection set-up required)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Hormonal assays of urine, feces, blood and tissue.

F. Experiment Controls:

In-flight: None.

Ground: Same as in flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Minimum on orbit requirements: rodent metabolic cages with activity and feeding monitors; data acquisition and storage system; waste material storage, animal telemetry system.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage, electrophysiology measuring equipment, work area, mass measurement device, ambient storage.

Experiment Identification Code: DE-b D = developmental; E = behavior/circadian rhythms

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Alan G. Lewis

Department of Oceanography
University of British Columbia
Vancouver, British Columbia
VAT 1Z4 Canada

Critical Question(s) Addressed:

DA-2, DC-1, **DE-1**, DE-2, DG-2, DG-4
(See p. A-6)

3. Experiment Title

The Importance of Gravity in Marine Food Chains

4. Purpose/Hypothesis

Besides causing particles to sink or to rise, reaction to gravitational forcing by primary producers and consumers affects species composition and energy transfer in marine food chains. Vertical migration of zooplankton also plays an important role in the transport of organic carbon away from the euphotic zone to deeper water. This may have an effect on atmospheric carbon dioxide uptake by the oceans.

5. Scientific Rationale/Rationale for Microgravity

This project is to evaluate the effect on orientation, substrate response and development in a tolerant marine copepod (*Tigriopus californicus*). Results of the study will help determine the role of gravity in substrate orientation by near-benthic crustaceans and vertical migration (ontogenetic and diel) by plankton.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

The effect of gravity on suitable marine organisms can best be studied under conditions of microgravity. These studies should include evaluation of orientation and movement, as well as development of the early life history stages since mobility and response can change with time during ontogeny.

C. Number and Type of Specimen:

Tigriopus californicus.

D. Measurements/Sample Handling:

Preflight: Preparation of experiment chambers.

In-flight: Video data collection. Preservation of specimens at various times.

Postflight: Preservation of remaining specimens.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Data analysis from video data. Analysis of preserved specimens.

F. Experiment Controls:

In-flight: 1-g centrifuge controls.

Ground: Ground-based controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g centrifuge(s) with the following properties: 1) each should handle 6 or more chambers (100-250 ml), 2) temperature control, 3) should be interfaced with video equipment that would allow continuous or semicontinuous viewing of the chambers; video equipment that: 1) can resolve living organisms in the 0.8-1.5 mm range. 2) has appropriate VCR recording equipment for storage of video. 3) has data transmission capability to Earth stations; equipment for specimen preservation while on centrifuge.

B. GBF Hardware Capabilities Required (on Orbit)

Small aquatic habitat (salt water), data storage, chemical fixation capability, ambient storage.

Experiment Identification Code: DE-c D = developmental; E = behavior/circadian rhythms

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Alexey M. Alpatov

123007 Moscow
Khoroshevskoye shosse
76-A

Critical Question(s) Addressed:

DE-1 (See p. A-7)

3. Experiment Title

Circadian Period as a Function of Gravity

4. Purpose/Hypothesis

The endogenous period of free-running circadian rhythms is altered in microgravity conditions.

5. Scientific Rationale/Rationale for Microgravity

The endogenous circadian period is a valuable physiological parameter: it determines the biological requirement of an organism for the duration of the day and its ability to cope with desynchronization. If this period is gravity-dependent, it will have serious implications for the design of work-rest schedules in space flight.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2: 30 days or longer

B. General Approach Description:

Monitor circadian rhythms of motor activity and body temperature in rats; activity and ERG in insects.

C. Number and Type of Specimen:

10 animals in individual cages. (rats, insects)

D. Measurements/Sample Handling:

Preflight: Same record as in flight or None.

In-flight: Collect data (motor activity, body temperature, ERG) in microgravity and on a 1-g centrifuge.

Postflight: Same as in flight or None.

E. Specific Sample Analysis:

In-flight: Data accumulation (recording).

Postflight: Time series analysis.

F. Experiment Controls:

In-flight: 1-g reference centrifuge is desirable.

Ground: Same as in flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Housekeeping of biological material, External Exposure Facility.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, insect habitat, electrophysiology measuring equipment, data storage.

Experiment Identification Code: DE-d D = developmental; E = behavior/circadian rhythms

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-R)

Critical Question(s) Addressed:

DE-2 (See p. A-7)

3. Experiment Title

Mating Behavior of Mutant Insects in Space

4. Purpose/Hypothesis

It is expected that adaptation to microgravity is manifested in less disturbed flying behavior and more efficient mating ability. Presumably such adaptation is the result of "learning".

5. Scientific Rationale/Rationale for Microgravity

This hypothesis can be tested by comparing the adaptiveness of normal wild strains and learning deficient mutants, e.g., dunce.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

The number of matings and mating behavior will be monitored in flight.

C. Number and Type of Specimen:

20 males, 30 females, wild; 20 male, 30 female dunce fruit flies (*Drosophila melanogaster*) (pupae).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Number of matings will be monitored by written record. Video monitor mating behavior.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Controls as per in-flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Insect habitat, video camera/VCR.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, data storage.

Experiment Identification Code: DE-e D = developmental; E = behavior/circadian rhythms

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Patricia Y. Hester

2. Author(s): Dr. Patricia Y. Hester

Department of Animal Sciences
Purdue University
West Lafayette, IN 47907

Critical Question(s) Addressed:

DG-3, DE-2 (See p. A-7)

3. Experiment Title

Microgravity Effects on Avian Reproduction

4. Purpose/Hypothesis

To determine if avians can reproduce in microgravity either through artificial or natural means.

5. Scientific Rationale/Rationale for Microgravity

Previous studies on space station MIR have shown that adult Japanese quail did not attempt to mate nor did the females lay eggs during a brief stay in microgravity. More intense investigations are needed to determine if these technological problems can be resolved.

Chicken, quail, and eggs can serve as a bioregenerative life support system. All are excellent sources of high quality protein that can be consumed by astronauts and space colonists.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 - up to 1 year

B. General Approach Description:

Either White Leghorn or Japanese quail will be exposed to microgravity immediately prior to sexual maturity. If results warrant, they will be maintained on the Space Station through a complete life cycle. Egg formation, feeding and mating behavior, and subsequent hatch of fertile eggs will be monitored.

C. Number and Type of Specimen:

10 White Leghorn pullets and 3 cockerels 18 weeks of age or 20 female and 6 male Japanese quail, 4.5 weeks of age.

D. Measurements/Sample Handling:

Preflight: Select and house birds for launch and transport.

In-flight: Although feeding and watering systems and behavioral observation will be automated, crew interaction will be needed daily to collect and set eggs in an incubator and perhaps to artificially inseminate hens if natural mating is not successful.

Postflight: The same data collected during flight will be collected post-flight on the returned animals to assess acclimatization.

E. Specific Sample Analysis:

In-flight: Collect and set eggs; collect semen and inseminate hens.

Postflight: Same as in-flight.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in flight experiment in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Habitat for adult birds, incubator for avian eggs with a hatching compartment. Half of the adults, eggs, and hatchlings should be submitted to 1-g force.

B. GBF Hardware Capabilities Required (on Orbit)

Avian adult habitat, avian hatchling habitat, data storage, work area.

Experiment Identification Code: DF-a D = developmental; F = aging

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-W)

Critical Question(s) Addressed:

DF-1 (See p. A-7)

3. Experiment Title

Effect of Spaceflight on Aging of Insects

4. Purpose/Hypothesis

Adult insects will experience accelerated aging in space.

5. Scientific Rationale/Rationale for Microgravity

This postulated life shortening in space is based on the fact that insects will use more oxygen (experience increased metabolic rate) because of uncoordinated flight in the absence of gravitational clues.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Video recording of insects in flight.

C. Number and Type of Specimen:

200 male fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Record survival data, maintain insects.

Postflight: Analysis of data collected in-flight.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: None.

Ground: Controls as per in-flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Insect habitats, video camera/VCR.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, data storage, -70°C freezer.

Experiment Identification Code: DF-b D = developmental; F = aging

1. Discipline: Developmental Biology

Reference SWG Member

(See Appendix H, Reference # 4)

2. Author(s):

(See Appendix H, Reference # 4: Experiment R/D-X)

Critical Question(s) Addressed:

DF-1 (See p. A-7)

3. Experiment Title

Effect of Spaceflight on Aging of Mutant Insects

4. Purpose/Hypothesis

The previously observed life shortening of insects in microgravity is the result of increased activity (and metabolic rate) due to uncoordinated flight.

5. Scientific Rationale/Rationale for Microgravity

The above hypothesis can be tested with the use of mutants which do not fly (vestigial wing, miniature, etc.). It is expected that these mutants will not experience life shortening relative to ground-based controls.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Video recording of insects in flight.

C. Number and Type of Specimen:

400 fruit flies (*Drosophila melanogaster*), 200 wild males, 200 mutants.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Record survival data. Maintain insects.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of data collected in-flight,

F. Experiment Controls:

In-flight: None.

Ground: Controls as per flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Insect habitats, video camera/VCR.

B. GBF Hardware Capabilities Required (on Orbit)

Insect habitat, data storage, -70°C freezer.

Experiment Identification Code: DG-a D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Ronald K. O'Dor

Dalhousie University
Department of Biology
Halifax, Nova Scotia, CANADA
B3H 4J1

Critical Question(s) Addressed:

DE, DG-4 (See p. A-7)

3. Experiment Title

Gravity and the Feeding Locomotor Dynamics of Small Aquatic Animals

4. Purpose/Hypothesis

To study the behavioral and physiological adaptations of small aquatic organisms (microplankton: 10 µm-1mm in length) to gravity. Reduced gravity will have a negative impact upon the ability of microplankton to swim and feed normally, thereby reducing sustained growth and reproduction.

5. Scientific Rationale/Rationale for Microgravity

A thorough understanding of aquatic production in both fresh and salt waters requires that the mechanisms by which organisms extract food energy from the environment are fully understood. Although previous studies suggest that gravity may influence swimming and feeding behavior of microplankton, adaptations of microplankton and their perception are unknown. Quantitative video observations of microplankton behavior together with a description of the ontogenetic development of gravity-sensing organs in several representative groups (from the Protozoa to the Crustacea) will provide information on the feeding success and survival of microplankton in a sustained microgravity environment.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

The swimming and feeding behavior of marine zooplankton will be monitored by three-dimensional automated tracking microscopy. The video data will be interpreted on the ground with the aid of a motion analyzer. Development of gravity-sensing organs in microgravity will be described histologically from specimens fixed in-flight.

C. Number and Type of Specimen:

Six species of microplankton representing three phyla and appropriate food source.

D. Measurements/Sample Handling:

Preflight: Measure size, number, specific gravity (via isopycnic centrifugation); fix for electron microscopy.

In-flight: Track and videotape organism motion in three dimensions, fix and/or freeze samples at three day intervals. Storage of computer-generated position data on non-volatile media.

Postflight: Estimate same variables as in preflight, analyze videotapes.

E. Specific Sample Analysis:

In-flight: Tracking should be automated, but manual intervention may be required.

Postflight: Kinematic analysis of videotapes for predator-prey interactions and feeding efficiency. Analysis of biochemical indices: lipid, oxidative enzyme activity, etc. (Epifluorescence microscope and clinical and ultracentrifuge required.)

F. Experiment Controls:

In-flight: 1-g control.

Ground: Parallel experimental treatments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Aquatic animal habitat (temperature, light regulation, filtration, etc.), three-dimensional video tracking microscope capable of following individual organisms in a 500 ml volume with 1µm resolution. A prototype of this microscope is currently being developed for space-based research by MPB Technologies Inc. of Montreal, under an arrangement with the Canadian Space Agency.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (salt water), compound microscope, chemical fixation capability, -70°C freezer, data storage, ambient storage.

Experiment Identification Code: DG-b D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Ronald K. O'Dor

Dalhousie University
Department of Biology
Halifax, Nova Scotia, CANADA
B3H 4J1

Critical Question(s) Addressed:

DE, DF-2, DG-1, DG-4 (See p. A-7)

3. Experiment Title

The Role of Gravity in Larval Bivalve Feeding and Locomotion

4. Purpose/Hypothesis

To study the role of gravity in feeding and locomotor behavior of bivalve larvae. A microgravity environment may significantly alter the mechanism by which bivalvae acquire food resources.

5. Scientific Rationale/Rationale for Microgravity

The processes by which zooplankton extract food from their environment are not clearly understood, but are believed to be governed by an interaction between drag and gravity. Earth-based experiments cannot separate the effects that these two components have upon feeding and locomotor behaviour. The high specific gravity of bivalve larvae suggests that they are particularly affected by gravitational forces. Removal of the gravity component should provide a better understanding of the relative contributions of gravity and drag to zooplankton feeding dynamics.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Bivalve larvae will be cultured under both microgravity and simulated 1-g conditions. Samples will be taken daily for post-flight analysis and assessment of physiological condition. Video record of swimming and feeding activity will be made, for estimation of feeding rates and food selection.

C. Number and Type of Specimen:

Approximately 10,000 sea scallop larvae (*Placopecten magellanicus*), fed a culture of the phytoplankton *Isochrysis galbana* (T-ISO strain).

D. Measurements/Sample Handling:

Preflight: Measure size, lipid reserves and specific gravity (via density gradient centrifugation) of larval samples.

In-flight: Remove daily samples, change videotapes, maintain constant food levels.

Postflight: Analysis of videotapes, assessment of larval conditions.

E. Specific Sample Analysis:

In-flight: Counts of algal concentration from videotapes.

Postflight: Motion analysis of videotapes, and larval samples. (Epifluorescence microscope and clinical and ultracentrifuge required.)

F. Experiment Controls:

In-flight: Counts of algal concentration from videotapes.

Ground: Motion analysis of videotapes, assessment of larval condition.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Marine animal habitat (temperature regulation, filtration, etc.), fixation facility, twin video camera assembly for three-dimensional reconstruction, recording capability.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (salt water), data storage, work area, chemical fixation capability, ambient storage.

Experiment Identification Code: DG-c D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. Ronald K. O'Dor

Dalhousie University
Department of Biology
Halifax, Nova Scotia, CANADA
B3H 4J1

Critical Question(s) Addressed:

DC-3, DD-1, DG-3, **DG-4** (See p. A-7)

3. Experiment Title

Selection of Suspension Feeding Aquatic Organisms for Culture in Microgravity Habitats

4. Purpose/Hypothesis

To select species that can feed efficiently on microalgae and complete their life cycles in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Many proposals for long-term space habitats include the use of water as mass for radiation shielding and the use of solar energy to drive natural ecosystems to recycle oxygen, nitrogen and carbon. Cultures of suspended microalgae are a logical way of combining these concepts, but efficient collection and conversion of microalgae to edible products is not a simple engineering problem. However, thousands of animal species have solved this problem and become quite edible. During the Space Station Phase 1, we proposed studies of the influence of microgravity on larval feeding; the long term follow up to this is to identify species that can complete their life cycles efficiently in space for future habitations.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 (up to 180 days)

B. General Approach Description:

Reproductive phase bivalves or crustaceans would be introduced into an aquatic or marine habitat linked to a recirculating algal culture system which would expose algae to natural sun light (or artificial light) under conditions previously shown to produce stable development and growth in 1-g. After induced spawning, larval survival and settlement and adult condition at the end of the exposure period would be monitored. Experiments could be set up to monitor species in isolation or competition.

C. Number and Type of Specimen:

Small numbers of adults of several species to be determined; possibly scallops and shrimp and other desirable food organisms with shorter generation times.

D. Measurements/Sample Handling:

Preflight: Adults transported at low temperatures.

In-flight: Manipulations (e.g. temperature shock) to induce spawning. Crew would count spawn before departure and collect and preserve juveniles on return.

Postflight: Determine survival and condition indices of preserved adults and juveniles, including biochemical condition.

E. Specific Sample Analysis:

In-flight: Larval counts (could be done from photos or video images on ground).

Postflight: Sizes, weights and biochemical composition (e.g. protein/lipid).

F. Experiment Controls:

In-flight: Several systems or partially isolated subsystems (possibly cages within the circulating system) could serve as comparative controls. Also algae only and/or adult-algae only controls would be desirable.

Ground: Duplicate closed systems to run in 1-g required for each experiment.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Closed marine/aquatic habitat with exposure to natural (preferably) light. Physical or image sampling system for larvae.

B. GBF Hardware Capabilities Required (on Orbit)

Medium aquatic habitat (salt water), [temperature controlled chamber], data storage, chemical fixation capability, refrigeration (4°C), ambient storage.

Experiment Identification Code: DG-d D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Marcelo E. Vasquez

Eye Radiation & Environmental Research Laboratory,
Columbia University
630 West 168th Street
New York, NY 10032

Critical Question(s) Addressed:

DG-5 (See p. A-7)

3. Experiment Title

Microgravity and Space Radiation Effects on Retinal Neuritogenesis

4. Purpose/Hypothesis

Changes in neuron differentiation are induced by microgravity, and radiation has been known to interfere with the neurite growth formation and elongation processes. A study is needed to determine the possible synergism of these two space factors on the development and plasticity of the CNS.

5. Scientific Rationale/Rationale for Microgravity

Several studies have suggested that microgravity alters neural cytoskeleton, neurotrophic interactions, signal transduction and gene expression, leading to an abnormal synaptogenic capability. Additionally, radiation can modify the formation and elongation of retinal neurites. Therefore, a study of the synergetic effects of microgravity and space radiations on the process involved in retinal neuritogenesis, will provide important information about neural functions and plasticity in space environment.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Neuroretinal explants will be cultured in-flight in both 0-g and 1-g conditions. Replicate samples will be taken daily and fixed. Neurite growth kinetics, histology and cytoskeletal protein expression will be assessed postflight.

C. Number and Type of Specimen:

60 explants for each experimental condition, each set of conditions is protocol dependent. Retinal explants from E6 chicken embryos cultured on plastic track detectors will be employed.

D. Measurements/Sample Handling:

Preflight: Preparation of retinal explants.

In-flight: Set up of explant cultures, daily removal of samples from each culture and fixation.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Morphometry of neurite length, density, growth index and explant area. Explant histology (EM, LM) and immunodetection of tubulin, actin, MAP, neurofilament and GAP43.

F. Experiment Controls:

In-flight: 1-g controls and, if available, radiation source.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

CO₂ cell incubator, 1-g CO₂ cell incubator, inverted microscope; laminar flow hood, 4°C refrigerator, fixation unit. Radiation source, if available.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, [temperature controlled chamber], data storage, work area, fluid handling tools, compound microscope, refrigeration (4°C), chemical fixation capability.

Experiment Identification Code: DG-e D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Basil Worgul

Director, Eye Radiation & Environmental Research
Laboratory, Columbia University
630 West 168th Street
New York, NY 10032

Critical Question(s) Addressed:

DG-5 (See p. A-7)

3. Experiment Title

The Possible Synergy of Microgravity and Space Radiation on Cataractogenesis

4. Purpose/Hypothesis

To determine whether or not ground based studies on radiation risk to integrated biological systems *in vivo* are relevant to space flight. Are there factors in flight which complicate the assessment of risk to the radiation environment?

5. Scientific Rationale/Rationale for Microgravity

Radiation cataract is among the most sensitive of *in vivo* late radiation effects known. It's amenability to radiation long-term non-invasive assay and the fact that it reflects genotoxic damage makes it an excellent paradigm to mutagenic damage in tissues less amenable to assay. Our recent work, using heavy ions, have shown that extremely low numbers of heavy ion hits can cause lens opacification. We believe flight experiments, preferably with an external radiation source and control for gravity (centrifuge), can be used to test if microgravity exacerbates the radiation risk in flight.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 or 3

B. General Approach Description:

Young, 60 g, Sherman rats will be flown and exposed to flight environment with and without gravity; with and without delivered radiation exposure.

C. Number and Type of Specimen:

Flight group will be 16-32 animals.

D. Measurements/Sample Handling:

Preflight: Scheimpflug and ocular assessment.

In-flight: None.

Postflight: Lifespan follow-up.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Lifetime cataract analyses using state of the art objective cataract assessment instrumentation.

F. Experiment Controls:

In-flight: 1-g controls and, preferably, a radiation source.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Animal containment and radiation source.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage.

Experiment Identification Code: DG-f D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. Gregory A. Nelson

Space Biological Sciences Group, MS 89-2
Jet Propulsion Laboratory
4800 Oak Grove Drive
Pasadena, CA 91109

Critical Question(s) Addressed:

DG-5 (See p. A-7)

3. Experiment Title

Tumorigenesis in Microgravity

4. Purpose/Hypothesis

Fluid redistribution, physiological adaptation and modification of growth factor environments inside mammals exposed to microgravity will modify the development of radiogenic tumors. If true the probability of tumorigenesis must be reevaluated and risk assessment values for humans adjusted accordingly. This represents a model to test the notion that radiation and microgravity will interact to adversely affect health.

5. Scientific Rationale/Rationale for Microgravity

Animals exposed to microgravity experience a variety of physiological changes including fluid redistribution, alterations in hormone regulation and immunological function as well as bone and muscle tissue loss. The set of changes represents a new homeostatic state which will be reproducible for microgravity environments and may serve as a significantly different environment for tumor initiation and development. Tail suspension models, *etc.*, can only mimic some of these environmental features.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3 (up to 16 months)

B. General Approach Description:

Mice irradiated with X-rays or gamma-rays prior to launch or during spaceflight would be incubated in microgravity vivaria for periods commensurate with the development of radiogenic tumors. In particular, tumors of the Harderian gland and mammary glands would be examined for alterations in kinetics attributable to microgravity exposure. Ideally, a 16-month microgravity exposure would be used but mice could spend early, middle or late phases of lifespan in 1-g or μ -g to detect sensitive periods. Mice would be sacrificed upon return to Earth for histological analysis and would be available for "parts program" utilization as well.

C. Number and Type of Specimen:

20 to 40 100-120 day old female B6CF1/An1 heterozygous mice with or without pituitary isografts at L-2 weeks.

D. Measurements/Sample Handling:

Preflight: Mice are irradiated (X-rays or gamma rays) to doses up to 5 Gray or 0 Gray as controls. Mice are aged together or in subgroups to give a single population (incubated 16 mo.) or sets of different aged animals (incubated 30-180 days).

In-flight: Mice are incubated in matched sets at 0-g and, if available, 1-g for several month periods. Routine physiological maintenance at 20°C until crew members recover the animals for return to Earth.

Postflight: Mice are given post-flight physiological examinations and aged as appropriate to allow adequate development of initiated tumors. Animals are sacrificed and characterized histologically for incidence, extent, and characteristics of tumorigenesis. Other tissues are made available for "parts program" use.

E. Specific Sample Analysis:

In-flight: Animals require routine inspection during flight.

Postflight: Incubation for completion of lifespan segments and physiological measurements performed as needed to assure vitality and to support parts program. Standard necropsy and histological analyses are performed following sacrifice.

F. Experiment Controls:

In-flight: In-flight centrifuge control should be performed with siblings of same vivarium lot.

Ground: Parallel ground controls with same lots should be performed.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Incubation at 20°C with and without 1 g centrifuge. Crew or automatic vivarium life support system activation/control.

B. GBF Hardware Capabilities Required (on Orbit)

Rodent weanling to adult habitat, data storage.

Experiment Identification Code: DG-g D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Barry Pass, D.D.S., Ph.D.

Faculty of Dentistry
Dalhousie University
Halifax, Nova Scotia
Canada B3H 3J5

Critical Question(s) Addressed:

DG-5 (See p. A-7)

3. Experiment Title

Effect of Densely Ionizing (HZE) Radiation on Dental Enamel

4. Purpose/Hypothesis

- (a) HZE ions produce long-lived free radicals in dental enamel.
- (b) HZE ions produce damage trails in the hydroxyapatite crystals of dental enamel.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have shown that (a) Sparsely ionizing radiation produces long-lived free radicals in the hydroxyapatite crystals of dental enamel. Detection of the free radicals by electron spin resonance provides a means of radiation dosimetry. HZE ions may produce a similar effect. (b) Nuclear fission tracks, or damage trails, are left by spontaneous and induced fission of uranium within crystals of hydroxyapatite. The number and size of these trails can be determined. Similar trails may be created by HZE ions. Consequently, dental enamel may be a useful in-vivo radiation dosimeter in space travel and simultaneously provide means of analyzing the nature of the HZE ions through their damage trails. These experiments can provide the basis for Space Station Phase 3 animal (mice) model studies that will supply in-vivo radiation dosimetry while monitoring the harmful effects of HZE ions on the same animals providing the dosimetry

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1: 16 days, Phase 2: 180 days

B. General Approach Description:

Phase 1 and 2: Human teeth, extracted in the course of normal dental treatment, and mice teeth, will be carried on a flight and allowed to be exposed to the ambient radiation. The concentration of induced free radicals and damage trails will be determined postflight.

C. Number and Type of Specimen:

Phase 1 and 2: 20 unblemished human teeth and 50 mice teeth. Also, two samples consisting of 1 gram of dental chips from each species. Teeth from the same individual will be used as available.

D. Measurements/Sample Handling:

Preflight: None

In-flight: None

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Phase 1 and 2: (a) Electron spin resonance studies of free radical accumulation in the dental enamel of the exposed teeth. (b) Measurements of the size and number of damage trails in the hydroxyapatite of the dental enamel of the exposed teeth.

F. Experiment Controls:

In-flight: Phase 1 and 2: Shielded teeth

Ground: Phase 1 and 2: Shielded teeth

7. Hardware Requirements

A. Minimum on Orbit Requirements

UF & MTC: Non shielding sample box, well-shielded sample box.

B. GBF Hardware Capabilities Required (on Orbit)

Ambient storage, data storage.

Experiment Identification Code: DG-h D = developmental; G = other

1. Discipline: Developmental Biology

Reference SWG Member

Dr. Richard J. Wassersug

2. Author(s): Dr. H. Chaim Birnboim

Ottawa Regional Cancer Centre
501 Smyth Road
Ottawa, Ontario
Canada K1H8L6

Critical Question(s) Addressed:

DG-5 (See p. A-7)

3. Experiment Title

Effects of Microgravity and Cosmic Radiation on Chromosome Stability in Mouse

4. Purpose/Hypothesis

The combination of low level high LET radiation and microgravity may affect the stability of chromosomes and influence double-strand DNA break repair processes in mammalian cells.

5. Scientific Rationale/Rationale for Microgravity

High LET radiation such as found in space is known to be very clastogenic, that is, to cause breaks in chromosomes. These chromosome "aberrations" are associated with increased risk of cancer. Double-strand breaks in DNA, which precede the chromosome breaks, are known to be repaired inefficiently, possibly because the broken ends move apart before repair can occur. There is no previous evidence concerning the effects (enhancement or interference) of microgravity on the "repair" of such double -strand breaks. It is proposed that this be tested.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

A mouse cell line developed in our laboratory for the purpose will be used. It has been genetically altered to eliminate the hypoxanthine phosphoribisyl transferase gene (HPRT) from the X-chromosome and to introduce a new HPRT gene onto an autosome. The loss of a whole chromosome or chromosome fragment containing the marker HPRT gene can be detected with very high sensitivity (as a cell resistant to the drug, 6-thioguanine.) The "spontaneous" rate of loss in the laboratory at 1g is from 0 to about 20 per million cells over a 14 day period.

C. Number and Type of Specimen:

Three cell lines would be used, 2 genetically altered and a control with the HPRT gene at its normal location on the X-chromosome.

D. Measurements/Sample Handling:

Preflight: Prepare cell stocks.

In-flight: None, if automated equipment is available.

Postflight: See E.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Scoring for rate of loss of chromosome containing the marker HPRT gene by determining the number of colonies of cells which have become resistant to the drug, 6-thioguanine. (Standard culture facility (CO2 cell incubator, laminar flow hood) required to determine frequency of chromosome loss.)

F. Experiment Controls:

In-flight: 1-g controls

Ground: Controls will parallel in-flight 1-g experiments.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Mammalian cell culture facilities. If an automated cell culture facility such as that being developed by Mortimer/Brown/Mclean is available, that would be ideal.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage.

Experiment Identification Code: PA-a P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. B.W. Poovaiah

Department of Horticulture
Washington State University
Pullman, WA 99164-6414

Critical Question(s) Addressed:

PA-1, PA-2, PA-5 (See p. A-10)

3. Experiment Title

Effects of Microgravity on Changes in Free Calcium Concentration in Germinating Seedlings

4. Purpose/Hypothesis

Gravity-induced alterations in the free calcium concentration is involved in root and shoot orientation.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have suggested that calcium is intimately involved in gravity signal perception and transduction. A novel molecular method has been developed to quantify changes in free calcium concentration in intact plants. We will have transgenic plants available for such an experiment. Therefore, a study examining the effects of gravity on free calcium level will provide valuable knowledge on gravity signal perception and transduction in plants.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Transgenic seedlings (F2 progeny of *Arabidopsis* or *Nicotiana*) carrying aequorin gene that can report changes in cytosolic free calcium concentration will be used to study the effect of gravity (1-g as well as under near 0-g conditions) on free calcium concentration. A luminometer will be used to monitor changes in light emission.

C. Number and Type of Specimen:

50 *Arabidopsis* or *Nicotiana* germinating seedlings.

D. Measurements/Sample Handling:

Preflight: Measure changes in free calcium concentration by monitoring light emission.

In-flight: Measure changes in free calcium concentration by monitoring light emission.

Postflight: Measure changes in free calcium concentration by monitoring light emission.

E. Specific Sample Analysis:

In-flight: Measure light emission.

Postflight: Measure light emission.

F. Experiment Controls:

In-flight: Variable-gravity centrifuge controls.

Ground: Ground-based controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, variable gravity centrifuge, luminometer.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, luminometer.

1. Discipline: Plant Biology

Reference SWG Member

Dr. Gerald F. Deitzer

2. Author(s): Dr. Harry Jan Swartz

Department of Horticulture
University of Maryland
College Park, MD 20742

Critical Question(s) Addressed:

PA-1 (See p. A-10)

3. Experiment Title

Shoot Organogenesis from Sweet Potato Roots: The Effects of Orientation on Auxin Metabolism

4. Purpose/Hypothesis

We have produced a technique to regenerate shoots directly (without intervening callus) from in vitro grown sweet potato roots. As roots can be stored in vitro for long periods of time and no plant growth regulators are used, this technique will allow a ready source of planting stock for prolonged flights without the risk of mutation or food contamination.

5. Scientific Rationale/Rationale for Microgravity

Our protocol involves rooting explants and inverting these roots on medium devoid of plant growth regulators. Non-inverted roots do not regenerate shoots; however, inverted roots produce negatively gravitropic roots (positive in the original orientation) which eventually cease growth -then shoots are formed within 12 weeks on >90% of these excised roots. The critical time for shoot induction, role of the negatively gravitropic roots and effects of gravity on hormonal balance are unknown in this developmental process. Auxins, responsive to gravitational forces, would seem to be involved in this response. Indeed, auxins (along with cytokinins) can stimulate shoot regeneration from root callus. We will try to determine a critical period for shoot induction prior to the proposed experimentation; however, the length of this inductive period could be measured in weeks.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and Phase 2

B. General Approach Description:

We propose investigating the effects of orientation and microgravity on the auxin physiology of in vitro shoot production from excised sweet potato root cultures. We will place inverted and non-inverted root explants on medium devoid of hormones and medium with a factorial combination (concentrations: 0, 1, and 10 μ M) of auxin: indole butyric acid; the antiauxin: clofibric acid; and the cytokinin: benzyl adenine. Cultures will be replicated under terrestrial and microgravity conditions. Previous research will attempt to determine a proper time, or inductive period, for experimentation. A "gut feeling" guess is the treatment would require 2 weeks. Shoot production will be monitored upon return to 1-g, or perhaps later, in microgravity. Another set of returning and terrestrial samples would be frozen at -70°C, sectioned and indole acetic acid concentrations would be determined using GC-MS. Control, 10 μ M clofibric acid and 10 μ M indolebutyric acid treated plants would be analyzed.

C. Number and Type of Specimen:

As this phenomena yields qualitative responses we do not have large numbers of replications except to produce tissue for hormonal determinations. 5 petri dish replicates of each of 27 plant growth regulator treatments x 4 orientation treatments would require 540 plates (100 mm diameter x 20 mm height - stackable, approximately 15 grams each [the size of plates could be reduced]). Another set of 90 plates would be required for the auxin analysis.

D. Measurements/Sample Handling:

Preflight: Cultures to be grown to their preinductive phase; all plant growth regulator treatments and explant manipulations will be performed.

In-flight: A subset of cultures will be inverted in flight. Samples for hormonal analysis will be frozen at two times (to be determined).

Postflight: Hormonal analysis samples will be kept frozen until delivered for extraction in Maryland. Cultures used to observe growth effects would require transport in their proper orientation at 20-25°C.

E. Specific Sample Analysis:

In-flight: None.

Postflight: The hormonal analysis samples will be ground along with a pentadeuterated indole acetic acid internal sample, partitioned, purified on HPLC, methylated with diazomethane and analyzed by gas chromatography-mass spectrometry.

F. Experiment Controls:

In-flight: In-flight controls (devoid of hormone), a subset inverted from lift-off orientation to control for g-forces developed during that period.

Ground: Duplicate experiments on the ground.

7. Hardware Requirements

A. Minimum on Orbit Requirements

A growth box held at 20 to 25°C and supplied with 30 $\mu\text{mol}/\text{m}^2$ /sec fluorescent light (continuous). Atmospheric pressure should be held at as nearly terrestrial as possible; oxygen tension may effect the response. A -70°C freezer would be required to hold hormonal analysis samples.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, (temperature controlled chamber), data storage, work area, dissection equipment, -70°C freezer.

Experiment Identification Code: PA-c

P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. Howard G. Levine

Department of Biochemistry and Cell Biology
State University of New York at Stony Brook
Stony Brook, New York 11794-5215

Critical Question(s) Addressed:

PA-1 (See p. A-10)

3. Experiment Title

Evaluation of Elevated Rates of Air Flow as a Countermeasure to the Detrimental Effects of Microgravity on Plant Chromosomes

4. Purpose/Hypothesis

To determine whether the imposition of elevated rates of air flow provide a countermeasure to the detrimental effects of spaceflight on plant chromosomes.

5. Scientific Rationale/Rationale for Microgravity

The tendency for higher rates of chromosomal aberrations to occur within plants subjected to spaceflight threatens the success of any microgravity-based CELSS project. Results from the CHROMEX experiment have hinted at the possibility of "wind" (air flow) induced "touch" effects providing a countermeasure to the detrimental effects of spaceflight on plant chromosomes.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, followed by Phase 3 for 6 mos.

B. General Approach Description:

Aseptically cultured plantlets of *Haplopappus gracilis* will be inserted into three aseptic Plant Culture Devices (PCD) and subjected to five different rates of air flow exchange. Two PCDs will be subjected to spaceflight, one of which will be centrifuged at 1-g, the third will be a ground control. All plant shoots will be severed upon planting into the PCDs, and the resulting roots which form during the experiment will be fixed in space (Phase 3 flights) or on Earth (Phase 1 flights) and examined post-flight.

C. Number and Type of Specimen:

Each PCD will consist of 5 replicate Plant Chambers (PCs), each capable of holding 20 plants with an ultimate shoot height of ca 15 cm and an additional 5-7 cm of space for the substratum in which root development will occur.

D. Measurements/Sample Handling:

Preflight: Experimental plantlets will be cultured aseptically and planted prior to lift-off (for Phase 1) or in space (for Phase 3).

In-flight: The PCD will continuously record temperature, light intensity and delivered airflow. Video records suitable for shoot growth data extraction taken each day. The Phase 3 experiment will require the ability to subject the plants to fixatives during flight.

Postflight: Phase 1 experimental plants will be fixed for cytological examination at recover. Phase 3 plants will have already been fixed in space.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Cytological examination (microscopy).

F. Experiment Controls:

In-flight: One PCD 1-g centrifuged control.

Ground: One PCD ground-based control. (One PCD and a walk-in growth chamber capable of precisely simulating the light, temperature, humidity and carbon dioxide parameters recorded within the Space Station environment in the vicinity of the PCDs.)

7. Hardware Requirements

A. Minimum on Orbit Requirements

Two PCDs and a centrifuge capable of providing 1 g to one of the PCDs. Each PCD should be capable of: both heating and cooling the plant environment; programmable light intensity and duration of exposure; continuous flow of 0.2µm filtered and either conditioned or completely defined gases through the apparatus. Video recording capability; Aseptic work area (PMC experiment).

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, work area (Phase 3), dissection equipment, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PA-d

P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Peggy A. Whitson

2. Author(s): Dr. Janet Braam

Rice University
Department of Biochemistry and Cell Biology
P.O. Box 1892
Houston, TX 77251-1892

Critical Question(s) Addressed:

PA-5 (See p. A-10)

3. Experiment Title

Microgravity Effects on Molecular Responses to Mechanostimulation in Plants

4. Purpose/Hypothesis

Mechanical stimuli such as touch, bending and gravity may share common signal transduction and response pathways. Alteration of normal gravitational conditions may affect the regulation of mechanostimulation-induced genes.

5. Scientific Rationale/Rationale for Microgravity

Expression levels of 5 genes (the TCH genes) in *Arabidopsis thaliana* are strongly up-regulated in response to a variety of mechanical stimuli. To determine the relationships between stimuli, such as wind, to the gravity stimulus, the regulation of these genes will be examined under micro-gravity conditions. Three of the 5 TCH genes encode calmodulin-related proteins; therefore, examining the microgravity effects on the regulation of these genes will also shed light on the role of gravity in calmodulin-related gene regulation. Transgenic plants that express an easily assayed reporter protein gene regulated through cis-regulatory regions of the TCH genes will simplify the analyses.

6. Approach

A. Flight/Experiment Duration: Space Station Phase I

B. General Approach Description:

Young plants will be grown in both 0-g and 1-g conditions. Replicate samples will be taken daily before and after mechano-stimulation (bending of plants). An aliquot of tissue will be frozen 30 minutes after stimulation and expression levels of the genes in these aliquots will be assessed post-flight by RNA analysis. In addition, aliquots will be photographed following submission in X-Gluc, the substrate for β -glucuronidase, the reporter protein, to determine the tissue localization of the molecular responses.

C. Number and Type of Specimen:

Two-week-old *Arabidopsis* plants will be used. Four pots of plants will be harvested daily, therefore 52 2-inch pots containing ~20 plants each will be required.

D. Measurements/Sample Handling:

Preflight: Prepare pots and sow seeds 2 weeks prior to flight.

In-flight: Water and fertilize every day.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Touch 2 pots of plants so as to bend plants back and forth 10X. Wait 30 min. Harvest and freeze 2 pots of plants: One touched; one untouched. For the other 2 pots (one touched, one untouched) harvest and submerge in X-Gluc, rinse, then photograph.

Postflight: Purify RNA from frozen samples and conduct Northern RNA blot analysis to assay TCH gene expression.

F. Experiment Controls:

In-flight: None.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Growth chamber area with fluorescent bulbs. Water and fertilizer, -80°C storage freezer, liquid nitrogen, if possible, for quick freezing.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, dissection equipment, -70°C freezer, snap/quick freezer, refrigeration (4°C), work area, ambient storage.

Experiment Identification Code: PA-e P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Lewis Feldman

2. Author(s): Dr. Lewis Feldman

Department of Plant Biology
University of California
Berkeley, CA 94720

Critical Question(s) Addressed:

PA-5 (See p. A-10)

3. Experiment Title

Tropisms In Plants

4. Purpose/Hypothesis

To determine the influence of microgravity on the perception and processing of various environmental stimuli.

5. Scientific Rationale/Rationale for Microgravity

The distinctive growth habit of plants is a result of the integration of a variety of environmental stimuli. Since many of these stimuli appear to modify/interact with gravity, studies conducted in the absence of gravity will allow us to understand the contribution of gravity to form and development in plants. Plants bend toward light (phototropism); they respond to touch by bending towards or away from the stimulus (thigmotropism); they grow towards moisture (hydrotropism). For these and other tropisms the processing of the stimulus results in a change in growth habit in relation to gravity, implying that the response of the plant to gravity is being modified by interactions of other tropisms. This work will examine the interactions/effects of gravity on both the perception and processing of various tropisms.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 10 days

B. General Approach Description:

Maize seedlings will be germinated in space or on ground and exposed to a variety of environmental stimuli. Growth habit will be recorded.

C. Number and Type of Specimen:

Maize seedlings (*Zea mays*) will be used. At least 10 plants should be used for each experiment.

D. Measurements/Sample Handling:

Preflight: Place seeds in pots.

In-flight: Water pots to start seeds growing. Photograph seeds in various habitats.

Postflight: Fix tissues for later analysis, depending on changes or alterations to the growth habit.

E. Specific Sample Analysis:

In-flight: None, but photograph extensively.

Postflight: Light microscopy on appropriate material. (Tissue embedding center, dissecting microscope, slide warmers, compound light microscope, and film processing facilities required)

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel inflight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Temperature controlled incubator in which plants can be exposed to a variety of environmental stimuli. Variable gravity centrifuge.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, (temperature controlled chamber), data storage, work area, ambient storage.

Experiment Identification Code: PA-f

P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Fred Sack

2. Author(s): Morris G. Cline

Department of Plant Biology
Ohio State University
Columbus, OH 43210

Critical Question(s) Addressed:

PA-5 (See p. A-10)

3. Experiment Title

Effects of Microgravity on Shoot-inversion Release of Apical Dominance in *Ipomoea nil*.

4. Purpose/Hypothesis

The bending down of the upper shoot causes the outgrowth of the highest lateral bud presumably due to gravity stress-induced restriction of growth in the inverted shoot. If the gravity stress of shoot inversion is prevented via a microgravity treatment, then apical dominance release should be inhibited.

5. Scientific Rationale/Rationale for Microgravity

Ground-based clinostat treatments in our laboratory have inhibited apical dominance release in plants with inverted shoots. Clinostating, however, is not a valid simulation of microgravity. Hence, to understand the role of gravity in apical dominance, microgravity treatments are needed.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

A microgravity and a 1-g treatment of plants with upright and inverted shoots.

C. Number and Type of Specimen:

40 plants in pots (10 in each of 4 groups).

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Bend down and secure apical shoots at fourth node immediately after microgravity condition begins. Measure shoot height and length of lateral bud at 4th node at that time.

Postflight: Measure shoot height and length of lateral bud at 4th node at end of microgravity treatment.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: 1-g basipetal and acropetal force controls

Ground: 1-g basipetal and acropetal force controls

7. Hardware Requirements

A. Minimum on Orbit Requirements

1 g centrifuge; growth chamber with controlled light and temperature.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, work area.

Experiment Identification Code: PA-g P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Gerald F. Deitzer

2. Author(s): Dr. Gerald F. Deitzer

Department of Horticulture
University of Maryland
College Park, MD 20742-5611

Critical Question(s) Addressed:

PA-5 (See p. A-10)

3. Experiment Title

Effects of Gravity on the Circadian Regulation of Light Quality Induced Changes in Flowering and Gene Expression

4. Purpose/Hypothesis

Microgravity may alter some component of the biological clock mechanism that regulates the temporal expression of many physiological and molecular events. This may result in altered responses to such environmental responses as the photoperiodic induction of flowering.

5. Scientific Rationale/Rationale for Microgravity

In order to maximize space and number of related results, a combined physiological and molecular approach will be conducted to establish whether gravity, or gravity pulses, in any way alter the phase of the circadian clock mechanism. Physiologically, the circadian change in the sensitivity of *Arabidopsis thaliana* floral enhancement by far-red (700-800nm) light will be studied. At the same time the effect gravity and gravity pulses on the light quality induced phase shifts in the circadian rhythm of chlorophyll a/b binding protein (*cab*) gene expression will be monitored.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1: 13 days

B. General Approach Description:

Arabidopsis thaliana plants will be grown for 4 days under 12 hour fluorescent photoperiods without an imposed gravity stimulus or supplemental narrow band light. On the fourth day, plants will be divided into 4 groups. Two groups will be given a 1 hour, 1.0-g gravity pulse, in either the middle of the light period or the dark period depending on the group. At the end of the 4 days the plants will all receive continuous fluorescent light for 72 hours. The 2 groups with gravity pulses and 1 group without will stay at microgravity and the remaining group will be placed on a 1.0-g centrifuge control. During this time, two samples from each group will be irradiated with far-red energy supplemented light for 6 hours starting every 3 hours. At the end of the 6 hour treatments, half of the irradiated plants will be harvested, frozen and stored at -80°C for postflight analysis of *cab* gene mRNA levels. The other half will return to 12 hour photoperiods, for 6 more days, before being harvested and fixed.

C. Number and Type of Specimen:

Arabidopsis thaliana ecotype Columbia seeds. This experiment will require 4 groups of samples with 2 replicates of each. The irradiation schedule will require 8 irradiations per day for 3 days. Each sample will consist of a single petri dish with 100 seedlings per dish. A total of 192 dishes will therefore be required.

D. Measurements/Sample Handling:

Preflight: Sow 100 seeds in nutrient agar (purified agar+ full strength Hoagland's #1 solution) in 200 petri dishes, let sit for 1 hour, then irradiate 15 min. with red light to insure uniform germination. Store at 4°C in the dark for up to 4 days through launch.

In-flight: Initiate experiment. Generate gravity pulses. Irradiate samples with far-red supplement light according to experiment protocol. Fix or freeze (liquid nitrogen) samples. Store frozen samples at -80°C.

Postflight: Keep all samples at -80°C or in fixative until time of analysis.

E. Specific Sample Analysis:

In-flight: None

Postflight: Weigh frozen samples, grind to a frozen powder and lyophilize to obtain dry weights. Phenol extract the powder and establish levels of cab mRNA by slot blot Northern hybridization using an available cDNA from *Arabidopsis*. Embed fixed samples in plastic and make longitudinal sections (using a microtome) to determine the degree of flowering attained by each sample. (Inverted stage phase contrast microscope required.)

F. Experiment Controls:

In-flight: Two separate, but identical on- and off centrifuge controlled environment chambers and a chamber containing both fluorescent and far-red lamps for the irradiation sequence.

Ground: Parallel experiments at normal gravity

7. Hardware Requirements

A. Minimum on Orbit Requirements

0-1 g temperature controlled centrifuge, 2 environmentally controlled growth chambers with cool white fluorescent lighting, Irradiation chamber with mixed cool white and Far-Red phosphor fluorescent lamps, -80°C freezer, liquid nitrogen supply, chemical fixation facilities.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, (temperature controlled chamber), data storage, fluid handling tools, work area, snap/quick freezer, -70°C freezer, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PA-h P = plant; A = gravity perception/transduction/response

1. Discipline: Plant Biology

Reference SWG Member

Dr. Arnold J. Bloom

2. Author(s): Dr. Arnold J. Bloom

Department of Vegetable Crops
University of California
Davis, CA 95616

Critical Question(s) Addressed:

PA-6 (See p. A-10)

3. Experiment Title

Microgravity Effects on the Development of a Tomato Mutant Deficient in Gravitropism

4. Purpose/Hypothesis

To examine whether the mutant tomato diageotropica that has an altered response to normal gravity has a similar response to microgravity.

5. Scientific Rationale/Rationale for Microgravity

Under normal gravity, exposure to ethylene will restore a normal gravitropic response to this mutant. Comparisons between the mutant and the wild type under microgravity with and without ethylene should indicate whether this mutant influences something fundamental to gravitropism.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Seeds of the mutant and wild type will be germinated in space and grown for a few weeks in the presence or the absence of low doses of ethylene and at micro- and normal-gravity. Plants would be harvested in space and fixed for ground based analysis.

C. Number and Type of Specimen:

Tomato seeds of the mutant and wild type, 24 each.

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Add water to seeds. One time thinning of germinated seeds to the 12 most vigorous plants of each genotype. Fixing the plants at the end of the experiment.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Morphological analysis of roots and shoots. (Image analysis equipment required for morphological quantification.)

F. Experiment Controls:

In-flight: Wild type and mutant with ethylene grown at normal gravity.

Ground: Growth experiments with wild type and mutant with and without ethylene.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, normal gravity centrifuge, tissue fixation equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, work area, dissection equipment, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-a P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Mary E. Musgrave

Louisiana State University Agricultural Center
Department of Plant Pathology and Crop Physiology
Baton Rouge, LA 70803

Critical Question(s) Addressed:

PB-1 (See p. A-10)

3. Experiment Title

Microgravity Effects on Seed Production

4. Purpose/Hypothesis

Microgravity-induced alterations in reproductive events will occur during a seed-to-seed cycle in space due to changes in the plant metabolism caused by an altered microenvironment.

5. Scientific Rationale/Rationale for Microgravity

Previous studies by the Soviets have suggested that seed production is impaired when plants are grown in reduced gravity. I propose a set of experiments to (a) confirm this observation and (b) understand the environmental effects on the plant which induce these alterations in reproductive performance.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2, 90 days

B. General Approach Description:

Plants will be planted in flight and grown through a full life cycle in microgravity. Growth stage should be monitored with video cameras for comparison with controls. Seed production and viability would be scored post-flight.

C. Number and Type of Specimen:

A rapid cycling crucifer would be used, such as *Arabidopsis thaliana*, or, preferably, *Brassica rapa*.

Approximately 80 plants would be expected.

D. Measurements/Sample Handling:

Preflight: Prepare germination chambers.

In-flight: None.

Postflight: Retrieve samples.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Germination assays; Video analysis.

F. Experiment Controls:

In-flight: 1-g controls

Ground: Controls will parallel 1-g controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant Growth Unit; 1 g Plant Growth Unit; Video camera capability within these units.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage.

Experiment Identification Code: PB-b P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Mary E. Musgrave

Louisiana State University Agricultural Center
Department of Plant Pathology and Crop Physiology
Baton Rouge, LA 70803

Critical Question(s) Addressed:

PB-1 (See p. A-10)

3. Experiment Title

Microgravity Effects on Plant Microenvironment Which Lead to Failure of Seed Production

4. Purpose/Hypothesis

Microgravity-induced alterations in reproductive events will occur during a seed-to-seed cycle in space due to changes in plant metabolism caused by an altered microenvironment.

5. Scientific Rationale/Rationale for Microgravity

Previous studies by the Soviets have suggested that seed production is impaired when plants are grown in reduced gravity. I propose a set of experiments to (a) confirm this observation and (b) understand the environmental effects on the plant which induce these alterations in reproductive performance.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3, 90 days

B. General Approach Description:

Plants will be planted in flight and grown through a full life cycle in microgravity. Plants physiological parameters would be monitored throughout the life cycle using small scale sensors situated around the plants. Harvesting in flight at 2 day intervals should confirm real-time measurements. Root systems would be quick frozen and stored for enzymatic analyses post-flight.

C. Number and Type of Specimen:

A rapid cycling crucifer would be used, such as *Arabidopsis thaliana* or, preferably, *Brassica rapa*.

Approximately 80 plants should be expected.

D. Measurements/Sample Handling:

Preflight: Prepare germination chambers.

In-flight: Sow seeds; sample every 2 days; quick freeze or fix samples; position sensors around plants and reposition as needed.

Postflight: Retrieve samples.

E. Specific Sample Analysis:

In-flight: Root zone oxygen probes; Gas exchange measurement system; Schlieren optical system.

Postflight: EM; Enzyme assays.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel 1-g controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant Growth Unit; 1 g Plant Growth Unit; Fixative handling area; -70°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, fluid handling tools, work area, dissection equipment, chemical fixation capability, snap/quick freezer, -70°C freezer, refrigeration (4°C).

Experiment Identification Code: PB-c P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. David Bubenheim

MS 239-7
NASA Ames Research Center
Moffett Field, CA 94035

Critical Question(s) Addressed:

PB-1, PB-3, PB-4, PB-5 (See p. A-10)

3. Experiment Title

Anthesis and Fertilization in Micro and Reduced Gravity

4. Purpose/Hypothesis

To determine micro and reduced gravity effects on development of pollen, including transfer, germination, pollen tube growth and orientation, and fertilization. Factors defining physiological state during reproduction provides the reference for events requiring polarity for successful completion of anthesis and fertilization.

5. Scientific Rationale/Rationale for Microgravity

The processes leading to seed yield and reproduction, sexual expression, exhibit a significant degree of polarity during several critical steps. This experiment would determine if gravity is required to provide the reference for that polarity, and if so provide an indication of the threshold range required to establish that polarity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, longer duration in later phases

B. General Approach Description:

Culture detached wheat spikes (heads) in test tubes (well established technique), place on orbit in appropriate treatments, remove samples (test tubes) every other day and fix entire spike or middle spikelets for return to ground.

C. Number and Type of Specimen:

25-50 spikes (heads: test tubes) per habitat

D. Measurements/Sample Handling:

Preflight: Grow wheat plants, remove spikes and put into detached head culture (approximately 30 day-old-plants), document uniformity.

In-flight: Treatment placement, regular sample removal and fixation (every other day), determine, on orbit, pollen viability on appropriate sample days (2nd and 3rd quarters of experiment).

Postflight: Examine fixed material, analyze on-orbit data.

E. Specific Sample Analysis:

In-flight: Method for on-orbit pollen viability determination TBD (several to choose from); fix spikes or spikelets at regular (daily or every other day) intervals.

Postflight: Microscopic analysis of returned, fixed material.

F. Experiment Controls:

In-flight: 1-g centrifuge treatment.

Ground: Simultaneous control, delayed to allow tracking of environmental factors actually experienced.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant habitats: 3 for reduced gravity, 1 for micro-g.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, fluid handling tools, dissection equipment, chemical fixation capability, work area, refrigeration (4°C).

Experiment Identification Code: PB-d P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. A.D. Krikorian

Department of Biochemistry and Cell Biology
State University of New York
Stony Brook, NY 11794

Critical Question(s) Addressed:

PB-2, PB-3 (See p. A-10)

3. Experiment Title

Cell Division and Microgravity

4. Purpose/Hypothesis

To determine whether cell division in a gravity altered environment is sustained qualitatively and quantitatively during organogenesis.

5. Scientific Rationale/Rationale for Microgravity

Work which has not enjoyed the benefit of a 1-g control in space shows altered cell division rates and modifications in karyological behavior in root cells. There is no way to substitute for microgravity on Earth. Clinostats are very inadequate models.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3; max. duration 90 days

B. General Approach Description:

Variable experiment duration will establish the "points" on a curve and unequivocally establish the facts.

Organogenic tissues or plant explants will be grown in a culture chamber on semi-solid medium and will be activated to make organs in microgravity at different points in time. 1-g controls will be carried out as well. Chemical fixations will be carried out after Tritium and P32 radioactive isotopes are added at the time of organ initiation. Organ samples will be surgically removed in space and chemically fixed; other samples will be quick (snap) frozen.

C. Number and Type of Specimen:

100 small (100 x 15 mm) culture chambers or equivalent. Aseptic cell culture or small organ tissue culture explant (aseptic-"germ"-free) test material.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Fix samples every 3 days for 20 samples or so. Photograph through chamber "windows" with manned intervention. Micro-g measurement every day for at least a few minutes. Introduction of radioactive phosphorous and Tritium. Quick freezing of samples. Preparation of radioactive tissues for further post flight analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Sample analysis on Earth.

F. Experiment Controls:

In-flight: 1-g centrifuge in early tests; later tests would be repeated at various levels of hypogravity.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g centrifuge; controlled environment culture chamber with automated capabilities for medium component control (pH, gas control, etc.), radioactive insertion and containment; capability to allow sampling of partial samples at computer software driven specified periods and to permit chemically "fixing" and/or "quick-freezing" (snap freeze). Culture apparatus should also permit light microscopy.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, (temperature controlled chamber), data storage, fluid handling tools, dissection equipment, snap/quick freezer, -196°C freezer, radioisotope handling equipment, work area, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-e P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Norman G. Lewis

Institute of Biological Chemistry
Washington State University
467 Clark Hall
Pullman, WA 99164-6340

Critical Question(s) Addressed:

PB-3 (See p. A-10)

3. Experiment Title

Alterations of Cell Wall Synthesis in Microgravity: Reaction Wood Formation

4. Purpose/Hypothesis

To determine if woody gymnosperms and angiosperms form compression and tension (reaction) wood in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Reaction wood formation appears to occur in response to an alteration in the (1-g) gravity perception by leaning wood stems. This alteration results in a cascade of biochemical events to compensate for the change; i.e., to restore the plant to an upright position. It now needs to be established whether this occurs in microgravity. Delineation is needed at the ultrastructural, chemical, and biochemical levels.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 and 3

B. General Approach Description:

To grow woody angiosperms and gymnosperms in microgravity while tethered, or displaced from an upright position, to determine if reaction wood formation occurs.

C. Number and Type of Specimen:

16-20 plant specimens of two species (gymnosperm and angiosperm) grown on liquid, semi-solid or solid media.

D. Measurements/Sample Handling:

Preflight: Initiate germination in sterilized plant growth chambers.

In-flight: Control of O₂/CO₂, nutrient uptake, pressure, vibration, light, temperature; measurements of growth and photosynthesis; use of radiochemical isotopes, plant tissue harvesting, sample dissection, microscopy, assays (enzymes, metabolites), freezing, freeze-drying.

Postflight: Electron microscopy, spectroscopy (mass, nuclear magnetic resonance, ultraviolet, infrared); enzyme assays, immunocytochemical analyses (enzymes and metabolites), chemical analyses.

E. Specific Sample Analysis:

In-flight: O₂/CO₂, humidity, pressure, vibration, light, temperature, growth characteristics (real-time downlinking via video), photosynthesis rate measurements, sample dissection and preservation, enzyme assays/metabolite production, radiochemical counting.

Postflight: Electron and light microscopy; mass, nuclear magnetic resonance, ultraviolet and infrared spectroscopy; enzyme assays; chemical analyses; immunocytochemical assays (enzymes/metabolites).

F. Experiment Controls:

In-flight: Use of variable-g apparatus, particularly 1-g.

Ground: Conditions "identical" to microgravity.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Fully automated controlled environment plant and cell growth facility for operator (astronaut) override, real-time (video) down linking; variable g capabilities; radioactivity sampling, sampling capabilities, light microscopy.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, (temperature controlled chamber), data storage, radioisotope handling equipment, work area, dissection equipment, compound microscope, dissecting microscope, snap/quick freezer, -196° C freezer, -70°C freezer, freeze dryer, ambient storage.

Experiment Identification Code: PB-f P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. Fred D. Sack

Department of Plant Biology
Ohio State University
Columbus, Ohio 43210

Critical Question(s) Addressed:

PA-3, PB-3, PB-6 (See p. A-10)

3. Experiment Title

Effects of Microgravity on the Differentiation of a Single Cell Specialized for Gravity Sensing

4. Purpose/Hypothesis

The tip cell of the moss *Ceratodon* grows upwards and has a specialized zonation of cell components. The purpose is to determine whether 1-g is necessary for normal differentiation.

5. Scientific Rationale/Rationale for Microgravity

Omnilateral stimulation (clinostat rotation) cannot substitute for microgravity in determining effects on differentiation. No data are available on whether microgravity affects the development of any single-cell-system that is specialized for gravity sensing in plants.

6. Approach

A. Flight/Experiment Duration: Space Station Phase I

B. General Approach Description:

Protonemal (filament) cultures will be maintained on agar in small, closed dishes and left in microgravity or placed in a 1-g flight centrifuge. Entire cultures will be fixed *in situ* and postflight.

C. Number and Type of Specimen:

32 dishes (less than 3 cm diameter and 1 cm deep)

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Fix samples at 4, 8 and 12 days. 4 dishes per treatment (0-g vs. 1-g) per day collected.

Postflight: Fix remaining 8 dishes on the ground.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Electron microscopy, confocal laser scanning microscopy.

F. Experiment Controls:

In-flight: 1-g flight centrifuge.

Ground: $\pm$ clinostat and $\pm$ 1-g centrifuge; equivalent mechanical perturbation and temperature fluctuations.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g centrifuge, constant temperature chamber (no need for light or for control of gas exchange), enclosure for injecting fixative into dishes.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, fluid handling tools, work area, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-g P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A. D. Krikorian

2. Author(s): Dr. V. Raghavan

Department of Plant Biology
Ohio State University
Columbus, Ohio 43210

Critical Question(s) Addressed:

PB-3 (See p. A-10)

3. Experiment Title

Role of Microgravity in the Life Cycle of a Fern (*Ceratopteris thalictroides*)

4. Purpose/Hypothesis

The life cycle of *Ceratopteris thalictroides* grown in the microgravity environment will be delayed with abnormal features.

5. Scientific Rationale/Rationale for Microgravity

To determine whether a fern with a short-life cycle will complete the event in a microgravity environment. To understand whether gametophytes grown in this environment experience any selective pressure in terms of percentages of male, female and bisexual.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2, 180 days

B. General Approach Description:

Spores will be allowed to germinate, form gametophytes, fertilization to occur and sporophytes to be formed in microgravity on the variable gravity centrifuge. At intervals, samples will be fixed for ground based observation.

C. Number and Type of Specimen:

Spores of *Ceratopteris thalictroides* cultured in suitable glass-containers.

D. Measurements/Sample Handling:

Preflight: Prepare cultures.

In-flight: Collect, fix samples.

Postflight: Examine spores at various stages of germination, gametophyte development and sporophyte development to determine pattern of germination, percentages of males, females, and bisexuals, and the pattern of sporophyte development. (Microscope required.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: As in D.

F. Experiment Controls:

In-flight: None.

Ground: 1-g control.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, variable gravity centrifuge, tissue fixation equipment.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, dissection equipment, chemical fixation capability, work area, refrigeration (4°C).

Experiment Identification Code: PB-h P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. A.D. Krikorian

Department of Biochemistry and Cell Biology
State University of New York
Stony Brook, NY 11794

Critical Question(s) Addressed:

PB-3, PB-4 (See p. A-10)

3. Experiment Title

Plant Somatic Embryogenesis in Microgravity

4. Purpose/Hypothesis

To determine whether developmental responses to the stimulus of gravity are limited to the formation of the primary wall during the first cell division of an embryonic cell.

5. Scientific Rationale/Rationale for Microgravity

There is no way to substitute for microgravity on Earth. Clinostats are very inadequate models.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3, max. duration 90 days

B. General Approach Description:

The experiment should be carried out under all flight scenarios (Space Station Phase 1, 2 and 3). Variable experiment duration will establish "points" on a curve and unequivocally establish the facts.

Embryonic cells or "egg surrogates" will be grown in microgravity at different points in time. 1-g controls will be carried out after radioactive carbon 14 is added at the time of activation. Other samples will be quick (snap) frozen.

C. Number and Type of Specimen:

60 small (50 x 100 x 15 mm) cell culture chambers or equivalent. Aseptic cell culture or small organ tissue culture explant (aseptic-"germ"-free) test material.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Sample fixation every six days for 10 samplings. Phase contrast microscopy through chamber "windows". Micro-g measurement every day for at least a few minutes. Introduction of 14°C. Removal of sample aliquots for quick freezing after centrifugation from the bathing liquid. Preparation of samples for post flight analysis.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: On Earth.

F. Experiment Controls:

In-flight: In flight 1-g centrifuge in early tests; later tests would be repeated at various levels of hypergravity.

Ground: None.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g centrifuge; controlled environment cell culture chamber with automated capabilities for medium component control, including pH and gas control etc., radioactive material insertion and containment; capability for automatic harvesting of samples at computer software driven specified periods and to permit chemically "fixing" them and/or "quick freezing" (snap-freeze). Cell culture apparatus with light microscopy.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, radioisotope handling equipment, work area, fluid handling tools, dissection equipment, temperature controlled laboratory centrifuge, snap/quick freezer, -196°C freezer, compound microscope, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-i P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Mary E. Musgrave

Louisiana State University Agricultural Center
Department of Plant Pathology and Crop Physiology
Baton Rouge, LA 70803

Critical Question(s) Addressed:

PB-1, PB-4 (See p. A-10)

3. Experiment Title

Microgravity Effects on Seed Production

4. Purpose/Hypothesis

Microgravity-induced alterations in reproductive events will occur during a seed-to-seed cycle in space.

5. Scientific Rationale/Rationale for Microgravity

Previous studies by the Soviets have suggested that seed production is impaired when plants are grown in reduced gravity. I propose a set of experiments to (a) confirm this observation and (b) understand the environmental effects on the plant which induce these alterations in reproductive performance.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Plants will be pre-grown before flight to a stage just before flowering. Plants would be harvested in flight at 2 day intervals and fixed for electron microscopy observations post-flight. Root systems would be quick frozen and stored for enzymatic analyses post-flight.

C. Number and Type of Specimen:

A rapid cycling crucifer would be used, such as *Arabidopsis thaliana*, or, preferably, *Brassica rapa*.

Approximately 80 plants would be expected.

D. Measurements/Sample Handling:

Preflight: Prepare pre-flowering plants.

In-flight: Remove samples every 2 days. Quick freeze or fix samples according to experiment protocol.

Postflight: Retrieve samples.

E. Specific Sample Analysis:

In-flight: None.

Postflight: EM; Enzyme assays.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel 1-g controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant Growth Unit; 1 g Plant Growth Unit; Fixative handling area; -70°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, fluid handling tools, work area, -70°C freezer, dissection equipment, snap/quick freezer, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-j P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. John G. Carman

Plants, Soils and Biometeorology Department
Utah State University
Logan, Utah 84322-4820

Critical Question(s) Addressed:

PB-1, PB-3, PB-4 (See p. A-10)

3. Experiment Title

Embryo Development From Detached Wheat Spikes in Microgravity

4. Purpose/Hypothesis

Null hypothesis: there will be no differences between spikes detached two days prior to anthesis and grown in detached spike culture in microgravity, and detached spikes grown under identical conditions but on Earth with regard to the following reproductive parameters: fertilization frequency (seed set), embryo axis length, scutellum length, hormone concentrations in embryos and ovular sap, and organic and inorganic nutrient concentrations in ovular sap.

5. Scientific Rationale/Rationale for Microgravity

Wheat embryos begin to develop at the "bottom" of the embryo sac about two to three days after pollination. There is published histological/cytological evidence that the zygote does not begin forming an embryo until after "globules" of dense endosperm form at the top of the embryo sac and coalesce by gravity around the zygote, which process provides nutrients and hormones that probably induce the zygote to undergo cell division. The proposed experiment would determine whether this coalescence process is indeed gravity-dependent. If it is, then it might be a major cause for poor seed set in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Wheat spikes at about two days prior to anthesis (pollination) develop normally and produce seed when they are detached along with about 10 cm of stem, and cultured such that the stem is inserted into a nutrient solution in a manner similar to a cut flower in a vase. Sets of spikes (about 50 total) will be detached and incubated in detached culture medium such that the closure between the stem and nutrient solution is tight. The nutrient solution will be contained in a bag that will collapse as transpiration occurs, thus preventing air to enter the nutrient solution volume. Spikes will be flown one day prior to anthesis such that anthesis, pollination and the initial stages of seed set occur in microgravity. Control spikes will be maintained on Earth.

C. Number and Type of Specimen:

Wheat spikes, about 50.

D. Measurements/Sample Handling:

Preflight: Minimal handling required.

In-flight: The ideal would be to kill and fix some spikes at daily intervals after anthesis to study normalcy of the fertilization process. Other spikes should be frozen to obtain hormone and nutrient concentrations of ovular sap.

Postflight: Immediate access for sampling by rapid freezing or killing and fixing would be required.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Samples will be analyzed for hormones (indirect noncompetitive ELISA and GC-MS), nutrients, and ultrastructure.

F. Experiment Controls:

In-flight: Centrifuge Facility to simulate 1-g.

Ground: Complete replication of cabin environmental conditions including simulation of in-flight gases and lighting required.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Lighting requirements are minimal in that the nutrient solution contains sucrose. The nutrient solution should be refrigerated to minimize bacterial or fungal contamination. The entire experiment should be sealed but equipped such that there is a constant flow of air through the bacterial air-vent orifices.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, (temperature controlled chamber), data storage, fluid handling tools, work area, chemical fixation capability, -70°C freezer, refrigeration (4°C).

Experiment Identification Code: PB-k

P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): B.V. Conger

Department of Plant and Soil Science
University of Tennessee
Knoxville, TN 37901

Critical Question(s) Addressed:

PB-5 (See p. A-10)

3. Experiment Title

Embryogenesis of Gramineae in Microgravity

4. Purpose/Hypothesis

To provide information on the influence of microgravity on embryo initiation, differentiation and development in a gramineous species.

5. Scientific Rationale/Rationale for Microgravity

The grass family, which includes the major cereals, is the most important in providing food for the world's human population. It is likely that long term space missions or colonization will include growing crops such as wheat, rice or corn. The proposed study is designed to investigate the processes mentioned in No. 4 above with a model system.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Orchardgrass (*Dactylis glomerata* L.) leaf segments will be cultured at 0-g and 1-g conditions. The basal 2 cm of the innermost leaf will be split down the midvein before cutting transversely into 3 mm length segments. Segments from one leaf half will be harvested and fixed for microscopic observation at 1, 3, 7, 10, 14 and 30 days. Those from the sister half will remain in culture to observe their response.

C. Number and Type of Specimen:

Orchardgrass leaf segments on petri plates, six for each time period at both 0-g and 1-g, 72 total plates.

D. Measurements/Sample Handling:

Preflight: Plate leaf segments onto solid (agar) medium in petri plates.

In-flight: Collect samples (leaf segments) at different times under sterile conditions and place in histological fixative.

Postflight: Embed tissues in paraffin, section, stain and conduct microscopic analyses. (Tissue embedding center, stereo (dissecting microscope), microtome, slide warmers, and compound light microscope required.)

E. Specific Sample Analysis:

In-flight: None.

Postflight: Light microscopy.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Temperature controlled incubator, variable gravity centrifuge, sterile transfer hood, stereo (dissecting) microscope.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, fluid handling tools, work area, dissection equipment, dissecting microscope, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-1

P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman Lewis

2. Author(s): Christopher S. Brown

Manager, Plant Space Biology

Mail Code BIO-3

Kennedy Space Center, FL 32899

Critical Question(s) Addressed:

PB-5 (See p. A-10)

3. Experiment Title

Microgravity Effects on Starch Metabolism and Growth in Germinating Soybean Seedlings

4. Purpose/Hypothesis

Transient starch formation in soybean cotyledons will be reduced in plants germinated and grown in microgravity. The biochemical mechanism for the alteration will be investigated.

5. Scientific Rationale/Rationale for Microgravity

Starch concentrations in plants exposed to microgravity are reduced. The germinating soybean is a model system which will allow determination of the possible mechanisms for the microgravity-induced alterations in starch, the impact on other carbon pools within the cotyledon and embryonic axis and the implication on early stages of plant growth.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Seeds will be watered and plant specimens will be maintained in microgravity and at various g-levels on the variable gravity centrifuge. At intervals, samples of plants will be harvested, and frozen or fixed for ground based analysis.

C. Number and Type of Specimen:

Soybean seeds, 144

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Add water to seeds, daily removal and freezing/fixation of samples.

Postflight: Maintain frozen samples.

E. Specific Sample Analysis:

In-flight: Photography.

Postflight: Starch and other metabolite concentrations and enzyme activities via UV/VIS spectrophotometry and HPLC.

F. Experiment Controls:

In-flight: Variable-gravity centrifuge controls.

Ground: Synchronous and asynchronous plant growth environmental controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, variable gravity centrifuge, tissue fixation equipment, -80°C freezer, liquid nitrogen freezing (or equivalent).

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, fluid handling tools, chemical fixation capability, -70°C freezer, snap/quick freezer, dissection equipment, work area, refrigeration (4°C).

Experiment Identification Code: PB-m P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Norman G. Lewis

Institute of Biological Chemistry
Washington State University
467 Clark Hall
Pullman, WA 99164-6340

Critical Question(s) Addressed:

PB-5 (See p. A-10)

3. Experiment Title

Determining Differences in Gene Expression in Plants Grown in Microgravity

4. Purpose/Hypothesis

To determine if changes in gene expression (induction or repression), and accompanying causal and ensuing biochemical consequences are influenced by microgravity.

5. Scientific Rationale/Rationale for Microgravity

Many differences have been noted in preliminary microgravity experiments regarding ultrastructural/chemical and biochemical alterations in plant growth and development. The *raison d'etre* for these changes can now be examined at the gene expression (induction/repression) level.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3

B. General Approach Description:

Grow three or four plant species (e.g., *Arabidopsis*, wheat, pine and *Leucaena leucocephala*) and examine gene expression for different tissues, organs, etc., at various stages of development. Plants will be grown on ground, in microgravity, and in microgravity at 1-g.

C. Number and Type of Specimen:

ca. 20 of each plant species, with dissection into various organs/tissues.

D. Measurements/Sample Handling:

Preflight: Initiate germination in sterilized plant growth chambers.

In-flight: Control of O₂/CO₂, nutrient uptake, pressure, vibration, light, temperature; measurements of growth and photosynthesis; use of radiochemical isotopes, plant tissue harvesting, sample dissection, microscopy, assays (enzymes, metabolites), freezing, freeze-drying.

Postflight: Construction of cDNA libraries and compare 'controls' to 'microgravity' grown tissues.

E. Specific Sample Analysis:

In-flight: None

Postflight: Determination of gene expression and repression using standard biochemical and molecular techniques.

F. Experiment Controls:

In-flight: Variable-g, 1-g on the space station.

Ground: Growth, dissection and analysis of plants (tissues/organs) under "identical" conditions.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Controlled environment culturing chambers (for growth under aseptic conditions), use of radioisotopes, sample dissection, cryogenic storage.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, radioisotope handling equipment, work area, dissection equipment, dissecting microscope, -70°C freezer, freeze dryer, refrigeration (4°C).

Experiment Identification Code: PB-n P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. Indra K. Vasil

Laboratory of Plant Cell and Molecular Biology
Department of Vegetable Crops
University of Florida
Gainesville, Florida 32611-0514

Critical Question(s) Addressed:

PB-5 (See p. A-10)

3. Experiment Title

Protoplast Fusion and Microgravity

4. Purpose/Hypothesis

To determine whether fusion of plant protoplasts in microgravity is different in altered gravity environments from what occurs on Earth.

5. Scientific Rationale/Rationale for Microgravity

Film surfaces in microgravity are profoundly different from what they are at 1-g. In normal gravity, the shape of the surface depends on fluid density etc. If gravity is absent, the stabilizing influence disappears, and the fluid responds to effects of changes in the geometry of the bounding walls. Novel cell associations should result.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3

B. General Approach Description:

Shortest experiment duration will be a few days; maximum duration will be on the order of 90 days. Variable experiment duration will establish the "points" on a curve.

Plant cells will be grown in a cell culture chamber in liquid nutrient medium under controlled conditions and will be reduced through automation to protoplasts in space at different points in time. 1-g controls will be carried out as well. Manned intervention is anticipated for several hours to "load" samples and monitor instruments. Real time photomicrography--phase, Nomarski will be carried out. Chemical fixations will be carried out after molecular probes and radioactive wall precursors (carbon 14; tritiated proline) are added. Other samples will be quick (snap frozen).

C. Number and Type of Specimen:

Cell culture chambers or equivalent. Aseptic cell culture or aseptic small organ tissue culture explant test material.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Phase contrast microscopy, video monitoring, and downlink capability through chamber. Samples fixed every six days for 10 samplings or so. Introduction of ^{14}C and ^3H -proline into cell chambers. Quick freezing and sample preparation for further post flight analysis.

Postflight: Maintain frozen samples.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Sample analysis on Earth.

F. Experiment Controls:

In-flight: 1-g centrifuge in early tests; later tests would be repeated at various levels of hypogravity.

Ground: Ground based controls will parallel in-flight 1-g studies.

7. Hardware Requirements

A. Minimum on Orbit Requirements

1-g centrifuge; controlled environment culture chamber with automated capabilities for medium component control (pH, gas control, etc.), radioactive insertion and containment; capability to allow sampling of partial samples at computer software driven specified periods and to permit chemically "fixing; and/or "quick-freezing" (snap freeze). Culture apparatus should also permit light microscopy.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, (temperature controlled chamber), data storage, fluid handling tools, radioisotope handling equipment, work area, dissection equipment, snap/quick freezer, -196°C freezer, -70°C freezer, compound microscope, chemical fixation capability, refrigeration (4°C).

Experiment Identification Code: PB-o P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Norman G. Lewis

Institute of Biological Chemistry
Washington State University
467 Clark Hall
Pullman, WA 99164-6340

Critical Question(s) Addressed:

PB-6, PC-1 (See p. A-10)

3. Experiment Title

Alteration of Cell Wall Synthesis in Microgravity: Phase I

4. Purpose/Hypothesis

To determine at the chemical, biochemical and ultrastructural levels, changes in plant growth and development in a microgravity environment.

5. Scientific Rationale/Rationale for Microgravity

Preliminary studies suggest that space-grown plants undergo considerable metabolic, physiological and structural changes when compared with their Earth-grown counterparts; i.e., it appears that cell walls are thinner, with typical cellulose microfibril orientation disrupted, and quantities of wall polymers (i.e., lignins, cellulose, hemicellulose, etc.) reduced. These alterations require an unambiguous explanation at the ultrastructural, chemical and biochemical levels.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 2 (or Phase 3); some Phase 1

B. General Approach Description:

To grow three plant species (i) monocot (such as wheat); (ii) a woody gymnosperm (e.g., pine); and (iii) a woody angiosperm (e.g., *Leucaena leucocephala*) and to determine how cell wall structure and organization is affected.

C. Number and Type of Specimen:

16-20 plant specimens of each species grown on liquid, semi-solid or solid media.

D. Measurements/Sample Handling:

Preflight: Initiate germination in sterilized plant growth chambers.

In-flight: Control of O₂/CO₂, nutrient uptake, pressure, vibration, light, temperature; measurements of growth and photosynthesis; use of radiochemical isotopes, plant tissue harvesting, sample dissection, microscopy, assays (enzymes, metabolites), freezing, freeze-drying.

Postflight: Electron microscopy, spectroscopy (mass, nuclear magnetic resonance, ultraviolet, infrared); enzyme assays, immunocytochemical analyses (enzymes and metabolites), chemical analyses.

E. Specific Sample Analysis:

In-flight: O₂/CO₂, humidity, pressure, vibration, light, temperature, growth characteristics via real-time downlinking via video, photosynthesis, sample dissection and preservation, enzyme assays/metabolite production, radiochemical counting.

Postflight: Electron and light microscopy; mass, nuclear magnetic resonance, ultraviolet and infrared spectroscopy; enzyme assays; chemical analyses; immunocytochemical assays (enzymes/metabolites).

F. Experiment Controls:

In-flight: Use of variable-g apparatus, particularly 1-g "controls".

Ground: Conditions "identical" to microgravity growth.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

Fully automated controlled environment plant and cell growth facility with capacity for perator (astronaut) override, real-time (video) down linking; variable g capabilities; radioactivity sampling capabilities, light microscopy.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, radioisotope handling equipment, work area, dissection equipment, compound microscope, dissecting microscope, snap/quick freezer, -196° C freezer, -70° C freezer, freeze dryer.

Experiment Identification Code: PB-p P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Arnold J. Bloom

2. Author(s): Dr. Arnold J. Bloom

Department of Vegetable Crops
University of California
Davis, CA 95616

Critical Question(s) Addressed:

PB-6 (See p. A-10)

3. Experiment Title

The Role of Turgor on Polar Growth Under Microgravity

4. Purpose/Hypothesis

The threshold pressure at which turgor produces growth diminishes under microgravity.

5. Scientific Rationale/Rationale for Microgravity

Plants under normal gravity require a minimum turgor pressure to initiate cell elongation and the consequent growth. This so-called threshold pressure varies with environmental parameters such as water stress and nitrogen availability. Polar growth and development may be altered under microgravity. These experiments would test whether the effects of microgravity are mediated through a change in threshold pressure.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Wheat seeds would be germinated in space and grown for a few weeks at micro- and normal-gravity. Leaves would then be attached to LVDT's to monitor growth, placed in pressure chambers to modify the plant turgor, and the relationship between these variables examined.

C. Number and Type of Specimen:

Wheat seeds, 24 in microgravity or normal gravity.

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Add water to seeds. Select healthy plant to attach to place in pressure chamber and attach to LVDT. Start automatic device to vary pressure and record changes in growth.\

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: None.

F. Experiment Controls:

In-flight: Plants grown and measured at normal gravity.

Ground: The same experiments at normal gravity.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, normal gravity centrifuge, pressure vessel able to sustain 10 bars pressure, valves to control pressure, LVDT's with 0.01 mm resolution, and appropriate recording devices.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, work area.

Experiment Identification Code: PB-q P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Norman G. Lewis

Institute of Biological Chemistry
Washington State University
467 Clark Hall
Pullman, WA 99164-6340

Critical Question(s) Addressed:

PB-7 (See p. A-10)

3. Experiment Title

Defining Structure and Function of Altered Gene Expression in Microgravity (Characterization of Clones)

4. Purpose/Hypothesis

To identify function of genes repressed or induced in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Differences in gene expression need to be translated into real biochemical, chemical and physiological events. This can be achieved by protein sequence deduction/determination and correlation with known enzymes, expressing the cDNA in other organisms, use of antisense RNA/cosuppression techniques, targeted gene disruption, etc. This will require plants grown (and transformed at 1-g), in microgravity, and at 1-g in microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1, 2 and 3

B. General Approach Description:

See section 5 above.

C. Number and Type of Specimen:

ca. 20-50 of each plant species, with dissection into various organs/tissues.

D. Measurements/Sample Handling:

Preflight: Initiate germination in sterilized plant growth chambers.

In-flight: Control of O₂/CO₂, nutrient uptake, pressure, vibration, light, temperature; measurements of growth and photosynthesis; use of radiochemical isotopes, plant tissue harvesting, sample dissection, microscopy, assays (enzymes, metabolites), freezing, freeze-drying.

Postflight: Analysis of tissues to determine effects of treatments described in section 5 above.

E. Specific Sample Analysis:

In-flight: None.

Postflight: See section 5 above.

F. Experiment Controls:

In-flight: Variable-g, 1-g on the space station.

Ground: Growth, dissection and analysis of plants (tissues/organs) under "identical" conditions.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Controlled environment culturing chambers (for growth under aseptic conditions), use of radioisotopes, sample dissection, cryogenic storage.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, radioisotope handling equipment, work area, dissection equipment, dissecting microscope, -70°C freezer, freeze dryer, refrigeration (4°C).

Experiment Identification Code: PB-r P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman Lewis

2. Author(s): William C. Piastuch

Plant Space Biology

Mail Code BIO-3

Kennedy Space Center, FL 32899

Critical Question(s) Addressed:

PA-2, PB-7 (See p. A-10)

3. Experiment Title

Gene Expression in Plants Subjected to Growth in Microgravity

4. Purpose/Hypothesis

These experiments will explore the hypothesis that differential gene expression, induced by a microgravity environment, is a contributing factor in the metabolic and structural changes found in plants grown for varying periods of time in spaceflight.

5. Scientific Rationale/Rationale for Microgravity

Plants respond to a wide variety of environmental conditions with altered or differential gene expression. Observed metabolic and structural changes in plants grown in microgravity suggest perturbation of normal molecular processes. Analysis of differential gene expression of plants grown under microgravity conditions will provide valuable insight on how these molecular changes would impact space plant physiology in terms of plant growth and productivity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Seedlings will be grown in-flight under microgravity and 1-g conditions. Plants will be photographed and harvested prior to landing and frozen (-196°C). Analysis of gene expression and protein production will be done postflight.

C. Number and Type of Specimen:

Plants will be a variety of dwarf wheat or *Arabidopsis thaliana*. Twenty-four wheat seedlings per treatment (2) or 200 *Arabidopsis* seedlings per treatment (2) would be required.

D. Measurements/Sample Handling:

Preflight: Planting and watering of seeds in the flight plant growth unit.

In-flight: Photography, harvesting and freezing of plants.

Postflight: Transfer of frozen material.

E. Specific Sample Analysis:

In-flight: None.

Postflight: mRNA isolation and quantification using various metabolic, structural, and stress-related gene probes. Protein analysis and identification of differentially expressed gene products.

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber (controlled lighting, humidity, temperature, and atmosphere) for microgravity and 1-g controls, camera, hood for harvesting, and a -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, work area, snap/quick freezer, -196°C freezer, dissection equipment.

Experiment Identification Code: PB-s P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman Lewis

2. Author(s): William C. Piastuch

Plant Space Biology

Mail Code BIO-3

Kennedy Space Center, FL 32899

Critical Question(s) Addressed:

PB-1, PB-7 (See p. A-10)

3. Experiment Title

Gene Expression in Plants Subjected to Multiple Generations of Growth in Microgravity

4. Purpose/Hypothesis

These experiments will explore the hypothesis that differential gene expression, induced by a microgravity environment, is the contributing factor in the metabolic and structural changes found in plants grown for varying periods of time in spaceflight. Multiple sexual generations of plants will result in some permanent genetic alterations affecting plant growth.

5. Scientific Rationale/Rationale for Microgravity

Plants respond to a wide variety of environmental conditions with altered or differential gene expression. Observed metabolic and structural changes in plants grown in microgravity suggest perturbation of normal molecular processes. Analysis of differential gene expression of plants grown under microgravity conditions will provide valuable insight on how these molecular changes would impact space plant physiology in terms of plant growth and productivity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Seedlings will be grown on-orbit under microgravity and 1-g conditions. Plant samples will be periodically photographed, harvested, and frozen for analysis on the ground. Seed from mature plants will be harvested, dried and replanted for a minimum of three generations. Analysis of gene expression and protein production will be done postflight.

C. Number and Type of Specimen:

Plants will be a variety of dwarf wheat or *Arabidopsis thaliana*. Fifty wheat plants per treatment (2) or 200 *Arabidopsis* plants per treatment (2) would be required.

D. Measurements/Sample Handling:

Preflight: Preparation of growth media, planting chambers, seeds, harvesting and storage containers, and nutrient solutions.

In-flight: Photography, measurement, harvesting and freezing of plants. Drying seeds and replanting in plant chambers.

Postflight: Transfer of frozen material.

E. Specific Sample Analysis:

In-flight: Measurement of plant growth, monitoring of plant media nutrient, water, and dissolved gas concentrations.

Postflight: mRNA isolation and quantification using various metabolic, structural, and stress-related gene probes. Protein analysis and identification of differentially expressed gene products.

F. Experiment Controls:

In-flight: 1-g controls

Ground: Controls will parallel in-flight experiments in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber (controlled lighting, humidity, temperature, and atmosphere) for microgravity and 1 g controls, camera; hood for harvesting, photography, and measurements; and a -196°C storage freezer.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, work area, snap/quick freezer, -20°C freezer, -196°C freezer, dissection equipment.

Experiment Identification Code: PB-t P = plant; B = reproduction/development

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman G. Lewis

2. Author(s): Dr. Barry McGurl & Dr. Ted Farmer

Institute of Biological Chemistry
Washington State University
Pullman, WA 99164-6340

Critical Question(s) Addressed:

PB-7 (See p. A-10)

3. Experiment Title

Gene Induction and Suppression in Microgravity

4. Purpose/Hypothesis

To identify and characterize plant genes which are induced or suppressed by gravity.

5. Scientific Rationale/Rationale for Microgravity

It is well known that microgravity-grown plant life forms differ in many ways from their Earth-grown counterparts, e.g., in size of organelles, in the deposition of macromolecular cell wall components, and in accumulation of other metabolites. This study will present a unique opportunity to define, at the gene level, the underlying reasons for such changes. It can be anticipated that the findings for such studies will provide a new dimension to biotechnological approaches in agriculture and forestry.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Arabidopsis seeds will be grown in space, harvested at maturity (roots, stems, leaves and flowers harvested) and frozen. The harvested material could be used to identify both genes which are expressed in response to gravity and genes which are suppressed by gravity. Genes expressed in root tips in response to gravity will be identified by screening a cDNA library synthesized using mRNA isolated from the root tips of plants grown in 1-g or synthesized using mRNA expressed in root tips grown in microgravity. Gravity-responsive genes and the proteins which they encode will be characterized by: 1) Using the cDNA to probe Northern blots of mRNA extracted from the experimental tissue. 2) Searching data banks with the cDNA sequence and deduced protein sequence. 3) Characterizing the protein encoded by the gravity-responsive gene by expressing the cDNA in bacteria or yeast, and raising antibodies against it. 4) Using antisense RNA technology to investigate gene function *in vivo*.

C. Number and Type of Specimen:

We consider the best model for this experiment to be *Arabidopsis thaliana* because of its short generation time and the ease with which subsequent genetic manipulations could be performed.

D. Measurements/Sample Handling:

Preflight: Plant seeds.

In-flight: Harvest roots, stems, leaves, and flowers. Freeze samples.

Postflight: Maintain frozen samples. Construct cDNA libraries from harvested material.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Identification of genes either induced or suppressed in microgravity and characterization of the gene products. (Large centrifuge, 2D-gel electrophoresis and related equipment required)

F. Experiment Controls:

In-flight: None.

Ground: 1-g controls. Plant a third box and using a large centrifuge, subject it to the high G-forces experienced during launch. Differences between these plants and those grown in 0-g should be due to the effects of 0 g and not the high G-forces of lift-off.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber with lighting, air, and water supplies.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, work area, snap/quick freezer, -70°C freezer, -196°C freezer, dissection equipment.

Experiment Identification Code: PC-a P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. David Bubenheim

MS 239-7

NASA Ames Research Center

Moffett Field, CA 94035

Critical Question(s) Addressed:

PC-1 (See p. A-10)

3. Experiment Title

Gas Exchange in Plants as Influenced by Gravity

4. Purpose/Hypothesis

To determine micro and reduced gravity effects on the gas exchange processes in plants: photosynthesis, respiration and transpiration. Reduced and microgravity may have primary or secondary affects on the physiological and physical processes which control rates of gas exchange in plants.

5. Scientific Rationale/Rationale for Microgravity

Knowledge of how gas exchange processes are influenced by gravity is essential for future reliance on plants as a primary life support system. Design and sizing of life support systems require identification of gas exchange rates in the environment they will operate. Food production, air revitalization and water purification all depend on predictable levels of gas exchange in plants.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and 3

B. General Approach Description:

Phase 1: Launch seedlings, place in appropriate treatment environments and measure gas exchange rates over flight period.

Phase 3: Germinate seeds in appropriate treatment environments and measure gas exchange over life cycle of plants.

Gas Exchange Measurements: Canopy and single leaf gas measurements are desirable (minutes to hours) allowing better measurement of exact leaf area. Experiment could be conducted with only the individual leaf measurement capability.

C. Number and Type of Specimen:

Phase 1: 20 dwarf wheat or soybean (both if only single leaf analysis)

Phase 3: 20 dwarf wheat or dwarf brassica.

D. Measurements/Sample Handling:

Preflight: Phase 1: Germinate, select uniform plants and plant habitat.
Phase 3: Pre-plant habitat.

In-flight: Observe system performance to be sure on line analysis systems are working, document plant developmental stages and sized. Place single leaf chambers on appropriate leaves over course of mission.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Automatic measurements of net photosynthesis, dark respiration and transpiration; estimates of leaf area index and mass accumulation should be included. Individual leaf gas analysis chambers should be placed on leaves for shorter term measurements.

Postflight: Mass and partitioning measurements.

F. Experiment Controls:

In-flight: 1-g centrifuge treatment.

Ground: Simultaneous control, delayed to allow tracking of environmental factors actually experienced.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant habitats: 3 for reduced gravity treatments, 1 for micro-g.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage.

Experiment Identification Code: PC-b P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Leland J. Cseke & Peter B. Kaufman

Department of Biology
The University of Michigan
Natural Science Building
Ann Arbor, MI 48109-1048

Critical Question(s) Addressed:

PC-1 (See p. A-10)

3. Experiment Title

Taxol Gene Expression in *Taxus canadensis* (Canadian Yew) Under Microgravity Conditions

4. Purpose/Hypothesis

To determine whether or not production of Taxol at 1-g on Earth is enhanced in space under micro-g conditions.

5. Scientific Rationale/Rationale for Microgravity

Taxol is a highly promising diterpene amide cancer chemotherapeutic agent which is needed in large amounts for clinical trials. However, attempts at synthesizing the compound have failed, and it is only attainable as a very small percentage (0.01%) in the needles and bark of the *Taxus* species. The mechanism behind the formation of Taxol is unknown, but in cases where the function of such secondary metabolites is known they serve to help the plant adapt to environmental (e.g. micro-g stress, low temperature stress) and biological stresses (e.g. predation).

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 and Phase 3 (30 day duration)

B. General Approach Description:

- 1) Ground-based control for exposure of plantlets to 1-g followed by HPLC separation and quantitation of Taxol.
- 2) Same in ground-based clinostat run at 10^{-5} -g.
- 3) Same in 1-g centrifuge in micro-g.
- 4) Same in micro-g.

C. Number and Type of Specimen:

4 plantlets of *Taxus canadensis* in 4 experimental conditions yielding 16 specimen total.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Freezing of plantlets in liquid nitrogen or -80°C freezer near the end of the flight duration.

Postflight: Maintain frozen plantlets. HPLC separation and quantitation of Taxol.

E. Specific Sample Analysis:

In-flight: None.

Postflight: All HPLC assays are done postflight. (HPLC/TLC equipment required.)

F. Experiment Controls:

In-flight: Centrifuge possessing two identical compartments: one run at 1-g for control, the other left to experience micro-g.

Ground: Two identical clinostats: one run to simulate micro-g (10^{-5} g), the other left to experience 1-g for 1-g ground-based control.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Liquid nitrogen freezer or -80°C freezer.

Two compartment centrifuge (available from U-Penn).

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, work area, -70°C freezer.

Experiment Identification Code: PC-c P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. Arnold J. Bloom

2. Author(s): Dr. Arnold J. Bloom

Department of Vegetable Crops
University of California
Davis, CA 95616

Critical Question(s) Addressed:

PC-1 (See p. A-10)

3. Experiment Title

The Balance Between Growth and Maintenance Respiration Under Microgravity

4. Purpose/Hypothesis

Maintenance respiration increases under microgravity.

5. Scientific Rationale/Rationale for Microgravity

Respiration can be divided into two compartments: maintenance and growth. Maintenance respiration varies with environmental parameters such as temperature, nitrogen availability, and elevated CO₂. Plants use gravity to perform many transport functions. Under microgravity plants might have to expend respiratory energy to conduct these functions; consequently, maintenance respiration might be greater under microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Wheat seeds would be germinated in space and grown for a few weeks at micro- and normal-gravity. CO₂ and O₂ exchange of the leaves would be monitored under various rates of nitrogen supply to vary plant growth rate. Both CO₂ and O₂ exchange are needed to determine whether respiration itself is being modified or whether different substrates are being catabolized. Attached LVDT's or photography would monitor leaf growth. Growth and maintenance respiration can be calculated from the relationship between gas exchange and growth.

C. Number and Type of Specimen:

Wheat seeds, 24 each in microgravity or normal gravity.

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Add water to seeds. Select healthy plant to attach to LVDT. Start automatic device to vary nitrogen supply and record changes in growth. Quick freeze plants at conclusion of experiment.

Postflight: None.

E. Specific Sample Analysis:

In-flight: Photography and CO₂ and O₂ analyses.

Postflight: Proximal analysis of plant tissue.

F. Experiment Controls:

In-flight: Plants grown and measured at normal gravity.

Ground: The same experiments at normal gravity.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, normal gravity centrifuge, CO₂ and O₂ analyzers with 2 ppm resolution at normal CO₂ and O₂ atmospheric concentrations, valves to modify rate of nitrogen supply, photography, LVDT's with 0.01 mm resolution, and appropriate recording devices.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, work area, dissection equipment, snap/quick freezer, -196°C freezer.

Experiment Identification Code: PC-d P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. A.D. Krikorian

2. Author(s): Dr. Peter B. Kaufman

Department of Biology
University of Michigan
Natural Science Building
Ann Arbor, MI 48109-1048

Critical Question(s) Addressed:

PC-1 (See p. A-10)

3. Experiment Title

Invertase Gene Expression in Graviresponsive Cereal Grass Pulvini Under Microgravity Conditions

4. Purpose/Hypothesis

To determine whether or not differential expression of the invertase gene that occurs during the gravitropic response in cereal grass pulvini at 1-g on Earth is blocked in space.

5. Scientific Rationale/Rationale for Microgravity

Clinostating pulvini nullifies differential expression of the invertase gene in graviresponding cereal grass pulvini. Does micro-g in space do likewise? Invertase is one of the key regulatory enzymes that is upregulated by GA3 & IAA differentially in graviresponding outshoot pulvini.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

- 1) Ground-based control analysis of invertase activity in top and bottom halves of graviresponding pulvini at 0, 3, 6, 12, and 24 hours.
- 2) Same in clinostat at 1-g.
- 3) Same in space on 1-g centrifuge.
- 4) Same in microgravity.

C. Number and Type of Specimen:

25 oat stem segments per time point at 5 time points with 4 experimental conditions yields 500 specimens.

D. Measurements/Sample Handling:

Preflight: None.

In-flight: Freezing tissue samples at indicated times; halving pulvini.

Postflight: Maintain frozen tissues for sample analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Run invertase activity assays. (Spectrophotometer required.)

F. Experiment Controls:

In-flight: 1-g centrifuge.

Ground: 1-g and clinostat (simulated 0-g or micro-g).

7. Hardware Requirements

A. Minimum on Orbit Requirements

Liquid nitrogen freezer or -80°C freezer; dark incubation chamber in which to gravistimulate stem squits (squits are 9 cm long and are placed between 2 glass plates and paper towels saturated with 0.1M sucrose).

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, work area, -70°C freezer, dissection equipment.

1. Discipline: Plant Biology

Reference SWG Member

Dr. Gerald F. Deitzer

2. Author(s): Dr. Bruno Quebedeaux

Department of Horticulture
University of Maryland
College Park, MD 20742-5611

Critical Question(s) Addressed:

PC-2, PC-6 (See p. A-10)

3. Experiment Title

Effects of Gravity at Various CO₂/O₂ Ratios on Plant Growth and Photosynthetic Partitioning

4. Purpose/Hypothesis

Microgravity may alter the partitioning of photosynthetically fixed carbon between starch and sucrose in the leaves of plants as well as the translocation of sucrose and other metabolites within the plants. These parameters are also affected by temperature and the relative ratios of the CO₂ and O₂ concentrations under which plants are grown. As the atmosphere aboard the Space Station may be different than that required to efficiently grow plants in microgravity, the interactions between temperature and various gas ratios will need to be established very early in the flight schedule.

5. Scientific Rationale/Rationale for Microgravity

Elevated CO₂ and diminished O₂ tensions, especially at lowered temperatures, lead to increased plant CO₂ fixation in leaves but this may not result in increased biomass production unless the fixed carbon is properly partitioned into sugar and translocated to other plant parts. These interactions are not completely understood on Earth and the effects of microgravity cannot be anticipated. This is especially true for translocation of assimilated carbon which is regulated biochemically at several different levels. An investigation of these biochemical regulatory pathways under microgravity will be essential to any attempt to successfully propagate plants during a Space Station mission of any duration.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

During the 13 day on-orbit mission, it is planned to simply establish a role for microgravity on the partitioning of fixed CO₂ between starch and sucrose in the leaf, and on translocation of sucrose and its partitioning between sucrose and starch in roots. This experiment will be conducted under normal CO₂ (350 µl/l) and O₂ (21%) tensions at normal temperatures (20-25°C). Seeds will be sown and imbibed for 4 days prior to launch and placed in controlled environments in orbit under either 1.0-g, in an on-orbit centrifuge, or under the reduced gravity on the station. Samples will be harvested by the crew on an alternate daily basis, divided into a leaf, shoot and root and frozen in liquid nitrogen for later analysis on the ground.

C. Number and Type of Specimen:

This experiment will utilize both a monocot (dwarf wheat) and a dicot (dwarf pea) and will include 3 seedlings of each at each harvest, which will be done on alternate days for 12 days, from each of the 2 conditions (1-g and 0-g). Thus, 12 seedlings will be harvested each day for a maximum of 72 seedlings that will be required at the beginning of the experiment (3 seedlings, 2 types, 2 conditions, 6 harvests).

D. Measurements/Sample Handling:

Preflight: Place 50 of each seed type in individual vials with a semipermeable cap on each in a black box (dark chamber at 20-25°C at 65-80% humidity) kept under constant temperature 4 days before launch.

In-flight: Seedlings will be placed in controlled environment chambers at day 1 or 2 in orbit, (1/2 in 0-g, 1/2 in 1-g centrifuge). Three seedlings will be removed every other day, separated into leaf, shoot (combined for monocots) and root, frozen in liquid nitrogen.

Postflight: All samples will be kept at -80°C after return for analysis.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Samples weighed, ground to a frozen powder and lyophilized to obtain dry weights. Levels of sucrose and starch will be measured, immunological techniques developed and ELISA tests of enzymes used to determine levels of key enzymes of carbohydrate metabolism. (ADC - Infra-Red CO₂ and H₂O Gas Analyzers; Hansatech O₂ Analyzer with fluorescent attachment; 2 EGC 8 sq ft Growth Chambers with HID Metal Halide Lighting; Sorvall Superspeed Refrigerated Centrifuge; Beckman multisample spectrophotometer; Millipore Immunoblotting System; and gas mixing system required.)

F. Experiment Controls:

In-flight: The on-board controls absolutely require the exact duplication of on- and off centrifuge controlled environment facilities. This will undoubtedly require two separate, but identical, controlled environment chambers.

Ground: Most ground based controls will be completed during the years preceding launch.

7. Hardware Requirements**A. Minimum on Orbit Requirements**

0-1.0g on board centrifuge within controlled environment; two identical controlled environment chambers (min. 2' x 4' growing space, equipped with four-400 W Metal Halide lamps each mounted external to the chambers and isolated from them by a hot mirror beneath each lamp (20-25°C+0.1°C); -80°C freezer, liquid nitrogen supply (ca. 100 lbs).

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, snap/quick freezer, -196°C freezer, dissection equipment, work area.

Experiment Identification Code: PC-f P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

A. D. Krikorian

2. Author(s): Dr. Allan H. Brown

Gravitational Plant Physiology Laboratory
University City Science Center
3401 Market St. Suite 350
Philadelphia, PA 19104-3323

Critical Question(s) Addressed:

PC-2 (See p. A-10)

3. Experiment Title

Reaction Wood Development in Relation to G-Forces

4. Purpose/Hypothesis

Reaction wood (RW), the localized development of morphologically distinct wood grown under tension or compression has been carefully studied only in a unit-g environment. The null hypothesis is that reaction wood (RW) formation will proceed quantitatively at all levels of g-force (from nominally zero (10⁻⁴g) to above one unit g) just as it does in earth's unit-g environment. It seems quite unlikely that the null hypothesis will be confirmed.

5. Scientific Rationale/Rationale for Microgravity

RW formation has been interpreted (a) as a weight induced stress response and also (b) as uniquely gravity dependent. According to (a) it is the direct result of the difference in "stress" on upper and lower sides of shoots (branches) that are not vertically oriented. Thus it is not uniquely a response to sensing the direction of the gravity vector, but according to (b) it is evidence of g-sensing by woody plant organs and not necessarily stress related. The literature on RW research can be cited to support either view and both (a) and (b) may be correct. Rationale for exploiting microgravity: It's not just μ g that is to be exploited: it's important to understand that it is the entire hypogravity range! The most straightforward way to relate RW formation to the g-force is to vary g, especially to test the effects of variable g in the hypogravity range which can be done unambiguously only in space.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 3

B. General Approach Description:

Woody plant seedlings will be grown under a range of chronically applied centripetal g-forces. The test seedlings will be oriented so that the force direction (in centrifuge coordinates, radially) will be applied transversely to the plant's or plant organ's longitudinal axis. Seedlings will grow in darkness. (Early on they will develop from food supplied by the seeds and for the duration of the test (about the last two weeks?) PAR light will be supplied by LEDs. These estimates need to be firmly established during experiment development. The growth progress will be monitored intermittently by time lapse video imagery (because it will not be possible to predict with confidence the pace of development in hypogravity). At a growth stage that would appear to coincide with an advanced stage of reaction wood development, the test would be terminated and the plant specimens would be fixed by a crew member trained in that procedure.

C. Number and Type of Specimen:

Pine seedlings probably will be good candidates for the first choice only because that genus has been well studied on earth. Numbers = TBD--a decision based on statistical and other considerations.

D. Measurements/Sample Handling:

Preflight: Seedlings can be started either on the ground or in orbit (preferably the latter).

In-flight: Seedlings can be installed in one (or more) centrifuges and allowed to develop in a TBS centripetal force environment until the test is terminated by cytological fixation of the seedlings for subsequent study on Earth.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Routine histological and cytological analyses by conventional methods.

F. Experiment Controls:

In-flight: In-flight controls.

Ground: Non-real-time ground controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Centrifugation apparatus (VSMDC units). Each VSMDC has two centrifuges that operate independently. The first experiment could be performed at μg (actually $10^{-4}g$); the second at $1.0g$. Both could be accommodated at the same time. (Subsequent tests at $0.10g$, $0.25g$, $0.50g$, $0.7g$). The space needed to accommodate one VSMDC is the equivalent of one Shuttle Middeck Double Locker.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, work area, dissection equipment, chemical fixation capability, refrigeration ($4^{\circ}C$).

Experiment Identification Code: PC-g P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. Arnold J. Bloom

2. Author(s): Dr. Arnold J. Bloom

Department of Vegetable Crops
University of California
Davis, CA 95616

Critical Question(s) Addressed:

PC-4 (See p. A-10)

3. Experiment Title

Ammonium and Nitrate Efflux Under Microgravity

4. Purpose/Hypothesis

Ammonium and nitrate efflux increases under microgravity.

5. Scientific Rationale/Rationale for Microgravity

Nitrogen is the mineral element that plants require in greatest quantity. Most plants acquire their nitrogen through root absorption of ammonium and nitrate ions. Because ion transport seems to depend on gravity, the normal fluxes of ammonium and nitrate are likely to be altered under microgravity. The proposed experiments would use the stable heavy isotope of nitrogen ^{15}N to monitor ammonium and nitrate influx and efflux under normal and microgravity.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Wheat seeds would be germinated in space and grown for a few weeks at micro- and normal-gravity. Plants would then be transferred to nutrient solutions containing 99.5% $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ for one hour. The plants would be removed, quick frozen, and stored. The solution would be saved for analysis on the ground for NH_4^+ and NO_3^- and $^{15}\text{N}/^{14}\text{N}$. Influx would be determined from the decline in $^{15}\text{NH}_4^+$ or $^{15}\text{NO}_3^-$ in the solution and the increase of ^{15}N in the plant tissue. Efflux would be determined from the increase in $^{14}\text{NH}_4^+$ or $^{14}\text{NO}_3^-$ in the solution.

C. Number and Type of Specimen:

Wheat seeds, 24 in microgravity or normal gravity.

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Add water to seeds. Select healthy plant. Transfer plants to 15N solution. Stop experiment by removing plants and quick-freezing them. Solution is saved.

Postflight: Preserve solution by adding acid. Maintain frozen samples.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Analysis of plants and solutions for N, NH₄⁺, NO₃⁻, and 15N/14N. (Isotope ratio mass spectrometer and N, NH₄⁺, and NO₃⁻ analyzers, required.)

F. Experiment Controls:

In-flight: Plants grown and measured at normal gravity.

Ground: The same experiments at normal gravity.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, normal gravity centrifuge, method for rapidly changing plant nutrient solutions.

B. GBF Hardware Capabilities Required (on Orbit)

Small plant habitat, data storage, fluid handling tools, work area, dissection equipment, snap/quick freezer, -196°C freezer.

Experiment Identification Code: PC-h P = plant; C = metabolism/transport

1. Discipline: Plant Biology

Reference SWG Member

Dr. Norman Lewis

2. Author(s): Christopher S. Brown & Thomas W. Dreschel

Manager, Plant Space Biology

Mail Code BIO-3

Kennedy Space Center, FL 32899

Critical Question(s) Addressed:

PC-4, PC-5, PC-6 (See p. A-10)

3. Experiment Title

Microgravity Effects on Water Use Efficiency and Nutrient Uptake in Plants

4. Purpose/Hypothesis

To examine the effect of microgravity on plant growth under varying root moisture and mineral nutrient environments.

5. Scientific Rationale/Rationale for Microgravity

Observed plant responses to water and nutrient stress and observed plant responses to the microgravity environment may be controlled by similar pathways which involve ethylene and ABA. The simultaneous application of these "stresses" may provide insight to the processes controlling the responses.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Seeds will be germinated and grown under various root water environments using a Porous Tube Plant Nutrient Delivery System as described by Dreschel and Sager (1989). This will allow accurate control of the water and nutrients available to the roots. Measurements of transpiration (water vapor), photosynthesis (carbon dioxide uptake) and ethylene production would be measured and sequential harvests performed for biomass, ABA, turgor, and nutrient status.

C. Number and Type of Specimen:

Wheat seed, 144

D. Measurements/Sample Handling:

Preflight: Prepare seeds for insertion into orbit.

In-flight: Maintain nutrient solution, weekly removal and freezing/fixation of samples.

Postflight: Maintain frozen samples.

E. Specific Sample Analysis:

In-flight: On-line CO₂, ethylene, and water vapor monitoring, nutrient solution volumes.

Postflight: Plant biomass, nutrient and water status, ABA.

F. Experiment Controls:

In-flight: Variable-gravity centrifuge controls.

Ground: Synchronous and asynchronous plant growth environmental controls.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber, variable gravity centrifuge, tissue fixation equipment, -80°C freezer, liquid nitrogen freezing (or equivalent), Porous Tube Nutrient Delivery System and associated hardware.

B. GBF Hardware Capabilities Required (on Orbit)

Large plant habitat, data storage, fluid handling tools, chemical fixation capability, dissection equipment, -196°C freezer, snap/quick freezer, work area, refrigeration (4°C).

Experiment Identification Code: PD-a P = plant; D = communities/microecology

1. Discipline: Plant Biology

Reference SWG Member

Dr. Charles Wade

2. Author(s): Dr. J.O. Kessler

Physics Department, Building 81
University of Arizona
Tucson, AZ 85721

Critical Question(s) Addressed:

PD (See p. A-11)

3. Experiment Title

Motility, Morphology and Oriented Locomotion of Algal Cells, Cultured in Microgravity

4. Purpose/Hypothesis

A. Cells cultured through many generations in microgravity will change in morphology, so as to lose their gravitational anisotropy (bottom-heaviness, center of mass offset).

B. i) Cells swimming in microgravity will exhibit alterations in oriented locomotion (e.g. phototaxis), especially after several generations. ii) Normal oriented locomotion requires both gravitational and physiological (sensory) alignment.

5. Scientific Rationale/Rationale for Microgravity

Oriented locomotion of swimming algal cells is guided by gravity, in association with other physical and physiological influences, e.g. shear and illumination. Only in microgravity can experiments be performed which can unambiguously decide the role of gravity as a mechanism for coaligning cells in association with other influences. Long-time residence in microgravity is the only way in which the influence of gravity on the reproduction and cell division processes can be eliminated for the case of free-swimming suspended cells. (Short space-flights and clinostats are ruled out).

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1 (followed up Phases 2 and 3)

B. General Approach Description:

Cells and cell colonies of various species (e.g. *Chlamydomonas*, *Carteria*, *Volvox*, *Peridinium*) will be cultured in flight and on the ground, under micro- and various g conditions. Liquid cultures are to be gently aerated, illuminated according to standard protocol, and kept at a standard temperature (e.g. 20°C). At set intervals, cells extracted from the cultures will be inserted into a motility/taxis chamber. Their movements and photo response will be recorded through a VCR/microscope system. The VCR tapes will be analyzed on the ground. The VCR data can be down-linked, and/or the physical tape retrieved.

C. Number and Type of Specimen:

Phase 1: *Chlamydomonas* and *Volvox* (2 cultures each/category)

Phase 2 and 3: These and additional cells/protocols as derived from the Phase 1 flights.

D. Measurements/Sample Handling:

Preflight: Choose and prepare cell stocks and media. Early preflight: Test methodology using ground control apparatus, KC 135, and shuttle flights.

In-flight: Set up cultures; periodically (e.g. 12 hours) remove samples and acquire vectorial locomotion data (concurrent ground control and space station runs at 1-g, 2-g and additional accelerations, if possible); freeze samples for later retrieval and analysis.

Postflight: Maintain frozen samples. Computer analysis of vectorial oriented/disoriented motile behavior; and morphometry/pattern analysis of retrieved frozen cells.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Derive probability distribution functions for cell speed, angle of trajectory relative to illumination and shear. Correlations of angle, speed and stochastic factors, as function of time in micro-g; division rates; morphometry: chloroplasts, pyrenoids, overall size, intracellular distribution of organelles. Location of daughter cells in colonials (e.g. *Volvox*) (Computer for analyzing cell motion statistics; electron microscope, standard microscope, and confocal sectioning capability required)

F. Experiment Controls:

In-flight: 2 cultures, each species at 1-g and 2-g (additional g levels if possible).

Ground: Same as in flight.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Illuminated growth chamber with aerator, microscope, CCD camera, VCR, taxis chamber, freezer space; specimen handling space-(glovebox), cell counting assay.

B. GBF Hardware Capabilities Required (on Orbit)

Cell culture apparatus, data storage, compound microscope, -70°C freezer, fluid handling tools, work area.

Experiment Identification Code: PD-b P = plant; D = communities/microecology

1. Discipline: Plant Biology

Reference SWG Member

Dr. Terry C. Johnson

2. Author(s): Dr. Peter Wong

Division of Biology
Kansas State University
234 Ackert Hall
Manhattan, KS 66506

Critical Question(s) Addressed:

PC-3, PD (See p. A-10)

3. Experiment Title

The Effects of Microgravity on the Cell-cell Interactions between *Rhizobium* and Legumes in the Formation of Nitrogen-fixing Root Nodules

4. Purpose/Hypothesis

The formation of legume root nodules is the result of cell-cell interaction between a prokaryote (*Rhizobium*) and a eukaryote (legume). Root nodules convert atmospheric nitrogen to ammonia and synthesize several specific proteins. The effects of microgravity on the formation of root nodules and on the synthesis of organic-specific proteins are largely unknown.

5. Scientific Rationale/Rationale for Microgravity

The sequences of root nodule formation have been well characterized morphologically and biochemically. The formation requires concerted cellular functions of the legume and the *Rhizobium*. The root nodule is an ideal system for the studies on the effects of microgravity on cell-cell interactions, metabolism, and protein synthesis.

6. Approach

A. Flight/Experiment Duration: Space Station Phase 1

B. General Approach Description:

Two bean (*Phaseolus vulgaris* L.) seeds will be planted in 50-ml culture tubes, which will contain a nitrogen-free plant nutrient solution solidified with agar. The planted seeds will be inoculated with about 10^6 *Rhizobium leguminosarum* Biovar. *phaseoli* cells. The culture tubes will be placed in plant growth chambers, which will be located in both 0-g and 1-g conditions. The structure of root nodules will be examined with light microscope and electron microscope. mRNA for leghemoglobin, glutamine synthetase (two nodule specific proteins synthesized by the legume), and nitrogenase (a protein synthesized by *Rhizobium*) will be isolated and quantified. The rate of nitrogen fixation will be measured.

C. Number and Type of Specimen:

Ten culture tubes containing planted bean seeds will be incubated in a plant growth chamber at 0-g and another ten tubes at 1-g.

D. Measurements/Sample Handling:

Preflight: Prepare culture tubes containing solidified plant nutrients. Prepare *Rhizobium* inoculant.

In-flight: Plant seeds and inoculate them with *Rhizobium*. Place culture tubes in growth chambers.

Postflight: None.

E. Specific Sample Analysis:

In-flight: None.

Postflight: Examine the structure of root nodules with light microscope and electron microscope. Quantify leghemoglobin, glutamine synthetase, and nitrogenase immunochemically. Measure the rate of nitrogen fixation by acetylene reduction assay and by ^{15}N incorporation. (Electrophoretic equipment, gas chromatograph, and mass spectrometer required.)

F. Experiment Controls:

In-flight: 1-g controls.

Ground: Controls will parallel in-flight experiment in 1-g.

7. Hardware Requirements

A. Minimum on Orbit Requirements

Plant growth chamber and 1-g plant growth chamber.

B. GBF Hardware Capabilities Required (on Orbit)

Temperature controlled chamber, data storage, work area, refrigeration (4°C).

SECTION II

SECTION II DEVELOPMENT

GENERAL PHILOSOPHY

The philosophy of the Space Station Biological Research Project (SSBRP) in soliciting reference experiments was to either supplement the first solicitation in areas that were not well represented or whose scope of hardware capability changed enough to preclude a majority of the original experiments. These areas included fish experiments for the Aquatic Habitat; insect experiments for the Insect Habitat; microbiology experiments for the Cell Culture Unit; reptilian egg and avian egg experiments that do not include hatching for the Egg Incubator; and experiments utilizing the Animal Biotelemetry System.

EXPERIMENT SOLICITATION

The experiment development process was initiated in June, 1995. Reference experiment solicitation packages were distributed by the SSBRP scientists to the international science community. Scientists with the appropriate background were identified either through current work presented at conferences or by an affiliation on the World Wide Web. A much broader audience was reached using this approach and was much more representative of an international collaboration as is embodied in the philosophy of the International Space Station era.

SOLICITATION PACKAGE

The SSBRP reference experiment solicitation package included a list of critical space biology research topics derived from the Cell, Developmental and Plant Space Biology Discipline Plans, a reference experiment template, and sample experiments to show the desired detail and content. In contrast to the first solicitation, scientists were not asked to design their experiments around three different types of flight opportunities. A complete solicitation package is included in Appendix I.

HARDWARE CAPABILITIES LIST DEVELOPMENT

The original GBF hardware capabilities list was based entirely on hardware needs identified by the reference experiments. The first draft of the GBF hardware capabilities list was developed during the July 1992 meeting of the GBF SWG at which time the identified hardware were prioritized by the GBF SWG based on science research priorities. During the evolution of SSBRP and the parallel realization that it would not be possible to provide all of the hardware on the original list, a new list was comprised abiding by the original priorities set forth by the GBF SWG yet staying within the realms of feasibility. A list of hardware definitions can be found in Appendix J. The complete original list is on pages 2-3,4.

Habitats Both 0-g and 1-g environments provided on-orbit

Advanced Animal Habitat-Centrifuge
Aquatic Habitat
Cell Culture Unit
Egg Incubator
Insect Habitat
Mouse Development Insert
Plant Research Unit

Laboratory Support Equipment

Passive Dosimeter
Dissecting Microscope
Specimen Labeling Tools
Refrigerated Centrifuge
Compound Microscope
Incubator
Small Mass Measuring Device
Slow Freeze Accessories
Dissection Equipment
Freezer (-80 degree C)
Quick/Snap Freezer (-196 degree C)
Refrigerator (+4 degree C)/Freezer (-20 degree C)
Fluid Handling Tools
Micro Mass Measuring Device
Battery Charger
Cleaning Equipment
Film Locker
Still Cameras
Video Cameras
Camera Locker
EM-Shielded Locker
General Purpose Hand Tools
Digital Multimeter
Digital Recording Oscilloscope

REFERENCE EXPERIMENT ORGANIZATION

The author's hypothesis, scientific rationale, general approach and hardware needs are described for each reference experiment. Experiment Identification Codes were developed which identify the subcategories of the critical questions posed by the Discipline plans that are addressed by each experiment. Identification Codes were assigned to the experiments based on the primary critical question subcategory addressed by the experiment. A list of the Identification Codes and critical question subcategory can be found on page 6-1.

DOCUMENT SUMMARY

A total of 66 experiments (3 cell and 63 developmental) were submitted by 49 different contributors. An author list is provided in Appendix K. On an experiment per habitat basis these included 6 for the Animal Biotelemetry System, 32 for the Aquatic Habitat, 3 for the Cell Culture Unit, 18 for the Egg Incubator and 7 for the Insect Habitat. Specimen types include cell cultures, insects, aquatic embryos, amphibians, fish (including Zebrafish), avian and reptilian eggs, rats and mice.

REFERENCE EXPERIMENT INDEX

The reference experiments are organized by discipline (Cell Biology and Developmental Biology) and gravitational biology critical question sub-category.

Each reference experiment has been assigned a three letter code, e.g. CA-a. The first two letters indicate the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The third letter makes the notation unique by indicating where the experiment is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments. CA-b is the second of the Cell Biology Gravity Sensing/Response experiments, etc. The reference experiments in Section II are also followed by (2).

The experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page.

Critical Question Sub-categories

(C) Cell Biology

- (CA)** Gravity Sensing/Response
- (CB)** Transduction/Response
- (CC)** Other

(D) Developmental Biology

- (DA)** Early Developmental Events
- (DB)** Later Developmental Events
- (DC)** Parturition/Postnatal Growth
- (DD)** Multigenerational Studies
- (DE)** Behavior/Circadian Rhythms
- (DF)** Aging
- (DG)** Other

CELL BIOLOGY EXPERIMENTS

Effects of Microgravity on F-actin Polymerization and Levels of Intracellular [cAMP] _i in Phagocytosing Immunocytes (blood cells) of Horseshoe Crab (<u>Limulus polyphemus</u>) and Hissing Cockroach	CA-a(2)	7-2
Effects of Microgravity and Space Radiation on Normal and Transformed Mammalian Cells.	CB-a(2)	7-4
Effect of Microgravity on the Encapsulating- and <u>de novo</u> Tubulin Synthesis Capabilities of Encapsulating (Pieces of Surgical Gut Sutures Implanted in the Abdominal Hemocoel of Cockroaches)	CB-b(2)	7-6

DEVELOPMENTAL BIOLOGY EXPERIMENTS

Microgravity Effects Upon the Embryonic Development of a Vertebrate Embryo.	DA-a(2)	7-8
The Effect of Microgravity in the Pattern of Process Formation During Mesenchyme Cell Migration in Starfish Embryos.	DA-b(2)	7-10
Microgravity Effects on Amphibian Central Nervous System (CNS) Development.	DA-c(2)	7-12
Effect of Gravity on Cell Lineages and Fates in Zebrafish Embryonic Development.	DA-d(2)	7-14
Embryonic Development of Goldfish After Artificial Fertilization in the Microgravity Environment.	DA-e(2)	7-16
Effect of Microgravity on Fish Sperm Motility Characteristics and Fertilizing Ability.	DA-f(2)	7-18
Analysis of Microgravity-induced Cellular Stress in Developing Zebrafish Embryos.	DA-g(2)	7-20
Analysis of Structural and Functional Modifications of a Cricket's Gravity Sensory System During Microgravity Exposure - Genetic versus Environmental Cues.	DA-h(2)	7-22
Effects of Microgravity on Parasite Development Inside a Diapausing Codling Moth.	DA-i(2)	7-24
Can Embryonic Reptiles Develop in the Absence of Gravity?	DA-j(2)	7-26
Effect of Weightlessness on Extra Embryonic Migration in Birds.	DA-k(2)	7-28
Effects of Weightlessness on Embryonic Positioning of Reptile Embryos.	DA-l(2)	7-30
Is Development of Avian Embryonic Axis Gravity Dependent?	DA-m(2)	7-32
Rat Skeletal Adaptation to Spaceflight and Readaptation to 1g.	DB-a(2)	7-34
The Role of AT _{1A} Receptors for Angiotensin II in Physiological Responses to Alterations in Gravity.	DB-b(2)	7-36
Monitoring <u>in vivo</u> Calcium Levels in Adult Rats in Microgravity.	DB-c(2)	7-38
Anti-oxidant Status in Freely Moving Mice in Microgravity.	DB-d(2)	7-40
Effect of Microgravity on Regional Blood Flow and Distribution.	DB-e(2)	7-42
Effect of Microgravity on the Ultrastructure of the Rat Neuromuscular Junction.	DB-f(2)	7-44
Hornet Comb Building Behavior in Space.	DB-g(2)	7-46

Embryological Development and Early Larval Development of Medaka.	DB-h(2)	7-48
Vestibular Hair Cell Regeneration in Microgravity.	DB-i(2)	7-50
Non-mitotic Hair Cell Regeneration in Microgravity.	DB-j(2)	7-52
Otoconial Development in Microgravity.	DB-k(2)	7-54
Effects of Microgravity on Cocoon Spinning and Metamorphosis of Insects.	DB-l(2)	7-56
Effect of Gravity on Wing Expansion in Newly Eclosed Tobacco Budworm Moths.	DB-m(2)	7-58
Effect of Microgravity on Chick Pre-Cardiac Differentiation and Heart Development.	DB-n(2)	7-60
Effect of Weightlessness on Hepatic Cytochrome P450 Function.	DB-o(2)	7-62
Effect of Weightlessness on Peripheral and Central Afferent Vestibular Innervation.	DB-p(2)	7-64
Effects of Microgravity on Intraocular Pressure (IOP) During Embryonic Eye Development.	DB-q(2)	7-66
Effects of Microgravity on the Morphology of the Vestibular End Organ.	DB-r(2)	7-68
Effects of Weightlessness on Heart Loop Formation and Late Cardiac Development.	DB-s(2)	7-70
Effects of Weightlessness on the Early Stages of Chick Heart Development.	DB-t(2)	7-72
Embryonic Organ Growth in a Weightless Environment.	DB-u(2)	7-74
Musculoskeletal Development in a Microgravity Environment.	DB-v(2)	7-76
The Role of Microgravity in Sex Determination of Embryonic Reptiles.	DB-w(2)	7-78
Using Microgravity to Examine the Structure and Function of Otolith Bones in the Inner Ear of Teleost Fishes.	DC-a(2)	7-80
Influence of Reduced Gravity on Buoyancy, Swimming, and Muscle Development in Fish.	DC-b(2)	7-82
Development of Gravity-sensing Organs in Long-term Microgravity.	DC-c(2)	7-84
Effects of Microgravity on Cardiovascular Function in Fish.	DC-d(2)	7-86

The Effect of Microgravity on Spermatogenesis in Lower Vertebrate.	DC-e(2)	7-88
Hormonal Profiles and Gamete Quality After Fish being Exposed to the Microgravity Environment.	DC-f(2)	7-90
Aquatic Habitat.	DC-g(2)	7-92
Effects of Microgravity on the Reproductive Biology of a Novel Vertebrate Model: A Self-Fertilizing, Hermaphroditic Fish (<u>Rivulus marmoratus</u>).	DC-h(2)	7-94
Development of <u>in vivo</u> Techniques to Study the Role of Ascorbic Acid (Vitamin C) in Ossification at Microgravity.	DC-i(2)	7-96
Neural Development and Adaptation in a Microgravity Environment.	DC-j(2)	7-98
Metabolic Consequences of Multiple Generation Exposure to Microgravity.	DD-a(2)	7-100
Microgravity and Space Radiation Effects on Stress Response in Insects.	DD-b(2)	7-102
Boundary Effects at the Gene-Gravity Interface on Population Character, Metabolic Function, and Energy Flow Patterns in a Eukaryote.	DD-c(2)	7-104
Effects of Microgravity on Reproductive Behavior in Fathead Minnows.	DE-a(2)	7-106
Effects of Microgravity on Orientation Response During Schooling Behavior in Zebrafish.	DE-b(2)	7-108
Stability of Fish with Different Body and Fin Forms Under Low Gravity.	DE-c(2)	7-110
Prenatal Development of Coordinated Behavior in Microgravity.	DE-d(2)	7-112
Detection of Radiation-Induced Mutations Using Transgenic Fish.	DG-a(2)	7-114
Development of the Static Vestibulo-ocular Reflex During Long-term Exposure of Fish Youngsters and/or Amphibian Tadpoles to Microgravity.	DG-b(2)	7-116
Gravity Effects in the Expression of the Ryanodine Receptor on the Developing Heart.	DG-c(2)	7-118
The Effect of Gravity on Tissue-specific Patterns of p53 Expression during Zebrafish Embryogenesis.	DG-d(2)	7-120
Development of <u>in vivo</u> Methods to Monitor Potential Mutagenic and Carcinogenic Risk Associated with Space Radiation.	DG-e(2)	7-122

The Effect of Gravity and Radiation on Tissue-specific Patterns of hsp70 Gene Expression During Zebrafish Embryogenesis.	DG-f(2)	7-124
Effects of Microgravity and Exercise on Bone and Muscle Morphology in Aquatic Vertebrates (Fish).	DG-g(2)	7-126
Feeding Day-old, Young, and Adult Quail in Microgravity.	DG-h(2)	7-128
Long Term Storage of Eggs Before Incubation.	DG-i(2)	7-130
Management of Day-Old Quail in Microgravity.	DG-j(2)	7-132

ANIMAL BIOTELEMETRY SYSTEM EXPERIMENTS

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Effect of Microgravity on the Ultrastructure of the Rat Neuromuscular Junction.	DB-f(2)	7-44

AQUATIC HABITAT EXPERIMENTS

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Aquatic Habitat	DC-g(2)	7-92

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CELL CULTURE EXPERIMENTS

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Effect of Microgravity on the Encapsulating- and <u>de novo</u> Tubulin Synthesis Capabilities of Encapsulating (Pieces of Surgical Gut Sutures Implanted in the Abdominal Hemocoel of Cockroaches)	CB-b(2)	7-6

EGG INCUBATOR EXPERIMENTS

Can Embryonic Reptiles Develop in the Absence of Gravity?	DA-j(2)	7-26
Effect of Weightlessness on Extra Embryonic Migration in Birds.	DA-k(2)	7-28
Effects of Weightlessness on Embryonic Positioning of Reptile Embryos.	DA-l(2)	7-30
Is Development of Avian Embryonic Axis Gravity Dependent?	DA-m(2)	7-32
Effect of Microgravity on Chick Pre-Cardiac Differentiation and Heart Development.	DB-n(2)	7-60
Effect of Weightlessness on Hepatic Cytochrome P450 Function.	DB-o(2)	7-62
Effect of Weightlessness on Peripheral and Central Afferent Vestibular Innervation.	DB-p(2)	7-64
Effects of Microgravity on Intraocular Pressure (IOP) During Embryonic Eye Development.	DB-q(2)	7-66
Effects of Microgravity on the Morphology of the Vestibular End Organ.	DB-r(2)	7-68
Effects of Weightlessness on Heart Loop Formation and Late Cardiac Development.	DB-s(2)	7-70
Effects of Weightlessness on the Early Stages of Chick Heart Development.	DB-t(2)	7-72
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The Role of Microgravity in Sex Determination of Embryonic Reptiles.	DB-w(2)	7-78
Prenatal Development of Coordinated Behavior in Microgravity.	DE-d(2)	7-112
Feeding Day-old, Young, and Adult Quail in Microgravity.	DG-h(2)	7-128
Long Term Storage of Eggs Before Incubation.	DG-i(2)	7-130
Management of Day-Old Quail in Microgravity.	DG-j(2)	7-132

INSECT HABITAT EXPERIMENTS

Analysis of Structural and Functional Modifications of a Cricket's Gravity Sensory System During Microgravity Exposure - Genetic versus Environmental Cues.	DA-h(2)	7-22
Effects of Microgravity on Parasite Development Inside a Diapausing Codling Moth.	DA-i(2)	7-24
Hornet Comb Building Behavior in Space.	DB-g(2)	7-46
Effects of Microgravity on Cocoon Spinning and Metamorphosis of Insects.	DB-l(2)	7-56
Effect of Gravity on Wing Expansion in Newly Eclosed Tobacco Budworm Moths.	DB-m(2)	7-58
Microgravity and Space Radiation Effects on Stress Response in Insects.	DD-b(2)	7-102
Boundary Effects at the Gene-Gravity Interface on Population Character, Metabolic Function, and Energy Flow Patterns in a Eukaryote.	DD-c(2)	7-104

SPACE STATION
BIOLOGICAL RESEARCH PROJECT
REFERENCE EXPERIMENTS

Experiment Identification Code: CA-a(2) C = cell biology; A = gravity sensing/response

1. Discipline: Cell Biology

2. Author(s): Dr. Ayodhya P. Gupta

Department of Entomology
J.B. Smith Hall, Cook College
Rutgers University, New Brunswick, NJ 08903-0231

Critical Question(s) Addressed:

CA-2, CA-1, CA-4

908-932-9459 Fax: 908-932-7229

3. Experiment Title

Effects of Microgravity on F-actin Polymerization and Levels of Intracellular [cAMP]_i in Phagocytosing Immunocytes (blood cells) of Horseshoe Crab (Limulus polyphemus) and Hissing Cockroach (Gromphadorhina portentosa).

4. Purpose/Hypothesis

To determine if the cytoskeleton organization of an arthropod immunocyte will be disturbed by gravity perturbation. Specifically, whether the normal process of cytoskeletal F-actin polymerization (from cytosolic G-actin) during the early stages of phagocytosis will be disrupted and the levels of [cAMP]_i depressed at zero gravity, resulting in immunosuppression of the phagocytic defense reaction.

5. Scientific Rationale/Rationale for Microgravity

Receptor-mediated phagocytosis is a major defense reaction in arthropods, and involves two blood cell (hemocytes) types, the granulocyte (GR) and/or plasmatocyte, collectively called, immunocytes. The most commonly reported changes during the initial phase of phagocytosis (the phase of recognition and targeting of the foreign antigen) are: formation of pseudopods, ruffling of the plasma membrane, and patching and capping of surface receptors, events that result in the reorganization of the cytoskeletal filaments, especially the F-actin microfilaments; both qualitative and quantitative changes occur in both F- and G- actin of the cytoskeleton. The F-actin polymerization is regulated by the intracellular second messenger, adenosine 3',5'-monophosphate [cAMP]_i. Would zero gravity affect these molecular events, and would these effects persist after return to unit gravity?

6. Approach

A. Experimental Duration: Inflight: one day; Postflight: 90 days.

B. General Approach

Description:

All morphological changes in phagocytosing immunocytes will be documented by immunofluorescence staining, semi-thin sectioning, and SEM procedures. Quantitative decrease in G-actin and concomitant increase in F-actin will be determined by DNase I inhibition and nitro-benzoxadiazol (NBD)-phalloidin staining procedures. [cAMP]_i levels will be determined, using cAMP[³H] assay kit (Amersham, Arlington Heights, IL).

C. Number and Type of Specimen:

20 centrifuge tubes containing 1×10^6 cells/ml in modified saline solution (MSS) and 20 tubes containing 1.0 - μ m diam, FITC-conjugated Fluoresbrite microspheres (FITC-FM) (1×10^7 FITC-FM/ml conc in MSS) (modified saline solution).

D. Measurements/Sample

Preflight: The above 40 tubes will be loaded in approved flight containers.

In-flight: To induce phagocytosis, some time during in-flight, each FITC-FM sample in the 10 tubes will be poured into the 10 tubes containing the immunocytes at 0-g; the other 10 cell-containing tubes will be similarly treated but at 1-g. All 20 treated tubes will be shaken at 15-min. intervals to facilitate phagocytosis for a period of 3 h, after which all

Postflight: tubes will be stored in a freezer until return to Earth.

E. Specific Sample Analysis:

In-flight:

Postflight: Determinations of F- and G- actin amounts and [cAMP]_i levels in the frozen phagocytosing immunocytes will be done on Earth.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: 1-g controls, same number as flight 1-g controls

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Material and technical help for F- and G-actin and [cAMP]_i determinations on earth and containers to hold the 40 centrifuge tubes and one crew member's assistance in mixing, shaking, and freezing the 20 experimental tubes in flight.

Ground: A similar set of requirements are needed for ground controls.

Experiment Identification Code: CB-a(2) C = cell biology; B = transduction/response

1. Discipline: Cell Biology

2. Author(s): Dr. Clive L. Greenstock

AECL, Radiation Biology & Health Physics Branch
Chalk River, ON Canada KOJ 1JO

Critical Question(s) Addressed:

CB-3

613-584-3311 ex. 6053 Fax: 614-584-4024

3. Experiment Title

Effects of Microgravity and Space Radiation on Normal and Transformed Mammalian Cells.

4. Purpose/Hypothesis

To determine whether the conditions of space flight alter the rate of malignant cell transformation in culture.

5. Scientific Rationale/Rationale for Microgravity

Any environmental perturbation can trigger biological changes that enable organisms to adapt and continue to flourish. Microgravity and/or space radiation will be tested for their ability to induce genetic and epi-genetic changes that influence the basal transformation frequency. In particular, the level and persistence of transformed foci and any disturbances in clonogenicity, plating efficiency and other unusual responses to culture conditions will be determined.

6. Approach

A. Experimental Duration: 1-90 days.

B. General Approach

Description:

Chinese hamster C3H 10T1/2 cells will be plated in culture flasks and allowed to incubate throughout the flight. After the flight, the surviving clones and the number of transformants per viable clone will be analyzed, utilizing standard staining and microscopic scoring techniques.

C. Number and Type of Specimen:

At least 20 flasks of C3H 10T1/2 cells, preirradiated to different radiation doses. Half to be put in 0-g and half in 1-g on the centrifuge.

D. Measurements/Sample

Preflight: Sample assembly, control measurements.

In-flight: Minimal.

Postflight: Return samples to the lab for scoring and analysis.

E. Specific Sample Analysis:

In-flight: None

Postflight: Measure total numbers of transformed foci per vehicle clone, and evaluate any effect of microgravity and/or space radiation.

F. Experimental Controls:

In-flight: None

Ground: Same as in-flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Sealed flasks to be installed in the GBF/Habitat and centrifuge.

Ground:

Experiment Identification Code: CB-b(2) C = cell biology; B = Transduction/Response

1. Discipline: Cell Biology

2. Author(s): Dr. Ayodhya P. Gupta

Department of Entomology
J.B. Smith Hall, Cook College
Rutgers University, New Brunswick, NJ 08903-0231

Critical Question(s) Addressed:

CA-2, CB-1, CB-3, CB-4, CB-5

908-932-9459 Fax: 908-932-7229

3. Experiment Title

Effect of Microgravity on the Encapsulating- and de novo Tubulin Synthesis Capabilities of Encapsulating (Pieces of Surgical Gut Sutures Implanted in the Abdominal Hemocoel of Cockroaches) Immunocytes of the Hissing Cockroach (Gromphadorhina portentosa).

4. Purpose/Hypothesis

It is hypothesized that the contact of the immunocytes with the implanted sutures will activate the inositol trisphosphate (IP₃) pathway--and will trigger transcription of tubulin mRNA during the nuclear phase of signal transduction. It will be determined if microgravity would affect the encapsulation and the de novo tubulin synthesis capabilities of the encapsulating immunocytes.

5. Scientific Rationale/Rationale for Microgravity

In previous study of encapsulation of pieces of implanted gut sutures in a cockroach, we found that in encapsulating (activated) immunocytes, there was a 7-fold increase in peripheral microtubules and a concomitant increase in nuclear pores. It is also known that in agonist-(e.g., Con A)- challenged immunocytes, the (IP₃) signal pathway is activated; we have found that in activated immunocytes of Limulus polyphemus, IP₃ receptors are indeed present on the plasma membrane and near the nuclear membrane. Thus, we expect that induced encapsulation of the implants will trigger de novo synthesis of tubulin mRNA by the nucleus. Would zero gravity affect these processes, and would these effects persist after return to unit gravity?

6. Approach

A. Experimental Duration: In-flight: 10 days; Post-flight: 90 days.

B. General Approach

Description:

Implantation of 1 mm-long sutures will be done onboard. Roaches will be first immobilized by exposing them to CO₂ for 5 min.; then pieces of gut suture will be implanted in the abdominal hemocoel through an incision in the membranous area with molten paraffin. Encapsulation of the sutures by the immunocytes will be allowed to continue for 10 days at both 0- and unit gravities in two groups of ten cockroaches each. In another group of ten cockroaches, implants will be inserted five days prior to the termination of the flight; these roaches will be maintained at zero gravity and returned to Earth with their implants, which will be retrieved after 5 more days.

C. Number and Type of Specimen:

30 live roaches in approved flight containers.

D. Measurements/Sample

Preflight: None

In-flight: The implanted sutures will be retrieved after 10 days by pulling them out with a forceps, and preserved (fixed), until return to Earth, in a mixture of 4% tannic acid and 2.5% glutaraldehyde (1:1) in 0.1M cacodylate buffer, containing 0.13M sucrose.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Freeze-fracture etching and electron microscopy (to study increase in peripheral microtubules and a concomitant increase in the number of nuclear pores) and measurement of tubulin mRNA by in situ hybridization techniques (to determine if de novo synthesis of tubulin occurred) will be done on Earth.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: 1-g controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Materials and technical help for the suggested procedures on earth and containers to hold 30 roaches, and one crew member's assistance for implantation and preservation of the encapsulated implants after 10 days.

Ground:

Experiment Identification Code: DA-a(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Robert K. Ho, Ph.D.

Department of Molecular Biology
Princeton University
Princeton, NJ 08544

Critical Question(s) Addressed:

DA-6, DA-5, DA-7, DB-1, DG-1, DG-4

3. Experiment Title

Microgravity Effects Upon the Embryonic Development of a Vertebrate Embryo.

4. Purpose/Hypothesis

To determine microgravity effects upon the localization of maternal determinants, the specification of embryonic axes, cell movements and organ formation in a vertebrate embryo.

5. Scientific Rationale/Rationale for Microgravity

The generation time of zebrafish, (*Danio rerio*) is relatively short (3 months) and the embryos are laid external to the mother. Because of these traits, very young zebrafish can be raised to breeding age in space. This would allow for the study of gravity effects upon the deposition of maternal determinant molecules in oocytes within the female. Both oocytes and embryos obtained from these females will be analyzed for pre- and post- natal effects of microgravity upon normal development.

6. Approach

A. Experimental Duration: 30-90 days.

B. General Approach

Description:

Juvenile zebrafish will be allowed to reach sexual maturity under microgravity conditions and then bred to obtain embryos. As zebrafish are a model system for vertebrate developmental studies, breeding and maintenance protocols for this organism are well established. Embryos will be analyzed by time-lapse video microscopy, some embryos will be fixed for later immunohistochemical and in situ gene expression studies on the ground.

C. Number and Type of Specimen:

10 male zebrafish; 20 female zebrafish.

D. Measurements/Sample

Preflight: None

In-flight: Some embryos will be observed under video time-lapse microscopy to analyze yolk cytoplasmic movements and cell movements during development.

Postflight: Fixed samples of embryos obtained under microgravity conditions will be processed for differentiation markers of axis specification, tissue specification and organ formation.

E. Specific Sample Analysis:

In-flight: Placement of embryos under a compound microscope with video camera hooked up to a ground-based telemetry link or a video recording device. Placement of various aged embryos into fixative solutions.

Postflight: None

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Breeding fish used in this experiment will be bred several months after being returned to normal gravity. Embryos obtained will be compared to experimental embryos. Some females may be sacrificed upon landing to obtain ovaries for analyses of oocytes.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Special module for care of aquatic organisms. Time lapse video microscopy station. Video uplink to time lapse microscopy station.

Ground:

Experiment Identification Code: DA-b(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Bruce J. Crawford

Faculty of Medicine / Department of Anatomy,
University of British Columbia
Vancouver, British Columbia V6T 1Z3 CANADA

Critical Question(s) Addressed:

DA-2, DB-2, DG-4, DG-3

3. Experiment Title

The Effect of Microgravity in the Pattern of Process Formation During Mesenchyme Cell Migration in Starfish Embryos.

4. Purpose/Hypothesis

During early starfish embryo development, mesenchyme cells "swim" through a gel filled blastocoel cavity by extending and retracting filopodia. Preliminary experiments show that this "swimming" process involves the polymerization and depolymerization of actin (and probably myosin) filaments. The purpose of this experiment will be to determine how microgravity affects the formation and retraction of these processes and thus the ability of these cells to migrate through the blastocoel.

5. Scientific Rationale/Rationale for Microgravity

Studies of the effects of microgravity on cell movements and the cytoskeletal events necessary for such movements in an intact embryo during embryogenesis should add to our knowledge of how gravity affects the way such movements are brought about and of the role of gravity in morphogenesis.

6. Approach

A. Experimental Duration: 10-14 days.

B. General Approach

Description:

Embryos reared to under conditions of microgravity will be fixed at critical time points. The preserved specimens will be returned to the ground where the positions of myosin and actin will be visualized with a confocal laser scanning microscope using either fluorescent antibodies against myosin or fluorescently labeled phalloidin. Other specimens will be prepared for SEM for detailed studies of the shapes of the cells and for TEM studies using colloidal gold labeled anti-myosin antibodies and phalloidin in order to study the details of the cytoskeleton. The results will then be compared with those of 1-g in flight controls and ground controls to determine the effects of exposure to microgravity.

C. Number and Type of Specimen:

Ripe female and male starfish and/or ripe females and frozen starfish sperm.

D. Measurements/Sample

Preflight: Spawn and fertilize eggs at 12C. 1h post fertilization, place at 5C. for transport to aquatic facility. Alternately, transport ripe females and "dry" sperm at 5C to space station, have personnel spawn female and fertilize eggs in microgravity.

In-flight: Either cryofix samples at 12h intervals or fix some in formalin (for immunological studies) and some in glutaraldehyde (for morphological studies).

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Analyze the details of the shape and arrangements of the cellular processes of the mesenchyme cells and compare them with that of 1-g and ground controls using laser confocal microscopy and scanning electron microscopy. Analyze the arrangement of actin and myosin using fluorescent labeled antibodies and laser confocal microscopy and transmission electron microscopy using colloidal gold labeled antibodies.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Controls to parallel in-flight experimentals and controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Equipment for spawning and fertilizing eggs. Small sea water aquaria for maintaining embryos. Equipment for fixation and/or cryofixation of the embryos. Fluorescence microscopes, laser confocal microscope, transmission and scanning electron microscopes.

Ground:

Experiment Identification Code: DA-c(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Anton W. Neff and George M. Malacinski

Medical Sciences Program
Indiana University, Bloomington, IN 47405

Critical Question(s) Addressed:

DB-1, DB-2, DA-7, DA-2, DG-1, DG-4

Anton (812) 855-7055 George (812) 855-1131

3. Experiment Title

Microgravity Effects on Amphibian Central Nervous System (CNS) Development.

4. Purpose/Hypothesis

The hypothesis to be tested is, under microgravity conditions the CNS of amphibian tadpoles "abnormally develops." Both our ground-based simulated weightlessness experiments on horizontal clinostats and flight experiments by others indicates that CNS development is affected by weightlessness. The hypothesis will be tested using molecular biology methods on amphibians (*Xenopus laevis*) raised in the aquatic facility.

5. Scientific Rationale/Rationale for Microgravity

The understanding of the early development of the nervous system in amphibians is currently being worked out at the molecular level. The emerging understanding of normal CNS development and the molecular markers that are necessary to investigate the phenomenon of the microgravity induced "abnormal development" of the amphibian CNS are available. The time is right to combine embryology, gravitational biology, and molecular biology to gain a deeper understanding the role gravity plays in the development of the CNS.

6. Approach

A. Experimental Duration: 1-8 days.

B. General Approach

Description:

Prepare gene-expression maps using molecular markers that are expressed in specific regions and specific times during CNS development. A staging series beginning with gastrulation through neurulation up to the feeding tadpole stage to correlate will be carried out to investigate the patterning of the CNS under microgravity conditions.

C. Number and Type of Specimen:

Microgravity fertilized Xenopus laevis embryos (approximately 800 embryos from a minimum of two spawnings - 5 stages of 40 each including 1-g centrifuged controls). Minimum of 8 adult females and 2 adult males.

D. Measurements/Sample

Preflight: Select females and males for flight. Isolate testes from males.

In-flight: Inject females with hormones, strip eggs, prepare sperm suspension from testes, fertilize eggs, sort fertile embryos, observe developing embryos and fix embryos at various stages for, histology, in situ hybridization, and immunocytology.

Postflight: Morphological and molecular analysis of nervous system-specific markers using standard morphometric histology, in situ hybridization, and immunocytology.

E. Specific Sample Analysis:

In-flight: Observational only with good record keeping.

Postflight: Standard tested morphometric histology and in situ hybridization, and immunocytology to localize nervous system-specific markers in fixed embryos from several stages.

F. Experimental Controls:

In-flight: In flight 1-g centrifuged controls that duplicate the microgravity set (one half of all the embryos from each spawning).

Ground: Duplicate of space flight protocol with independent spawnings using horizontal clinostat rotation to simulate the 1-g centrifuge space controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Facilities to house Xenopus (females and males) and embryos; dissecting microscope to observe embryos; "in flight" 1-g centrifuge to incubate developing embryos are required.

Ground: Ground based equipment and facilities necessary for histological and molecular analysis of experimental embryos.

Experiment Identification Code: DA-d(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Zvi Kam

Molecular Cell Biology, Weizmann Institute of Science
REHOVOT 76100, Israel

Critical Question(s) Addressed:

DA-6, DA-2, DA-7, DG-4

972-8-343473 Fax: 972-8-469713

3. Experiment Title

Effect of Gravity on Cell Lineages and Fates in Zebrafish Embryonic Development.

4. Purpose/Hypothesis

Following fertilization the zebrafish egg establishes asymmetric distribution of yolk and cytoplasm, causing cells to segregate and divide on top of the yolk sac. Still body axes were shown not to be strictly determined by the orientations of the first cleavages. Later during epiboly, the embryo rotates to pose reproducibly the body axes. The purpose would be to watch body axes definition in absence of gravity.

5. Scientific Rationale/Rationale for Microgravity

Cell fates have been assigned to regions of the embryo, but deterministic lineages were not found. If small variations in orientation of the fertilized egg cause nondeterministic lineages, by affecting polar segmentation of cellular components for example, its absence may allow to obtain a reproducible lineage tree for vertebrates. Also in-situ hybridizations and whole-mount immunolabeling would reveal patterns of RNA and protein expression, cytoskeleton organization, etc. Polar patterning reflect embryonic axes formation.

6. Approach

A. Experimental Duration: 1-3 days.

B. General Approach

Description:

Embryos produced by mating female and male fish. Single eggs, matrices of eggs mounted in a chamber under the microscope, and digital movies acquired.

C. Number and Type of Specimen:

20 males and 20 females adult zebrafish separated in 20 liters of fresh water at 28 deg. cent (20C).14/8 hours light/dark cycle. Mating aquarium.

D. Measurements/Sample

Preflight: Select fertile fish from clone.

In-flight: Mate females and males. Follow live, and fix embryos at various developmental stages for whole-mount in-situ analysis (hybridizations, Immunofluorescence) and genetic/biochemical analysis of whole embryos or dissected tissue extracts. Typical fixatives are paraformaldehyde and glutaraldehyde. Fixed embryos kept long at 4C

Postflight: before staining/hybridization. Extracts kept at -20C.

Postflight: Fixed samples staining, hybridization. Extracts analysis (gels, blots).

E. Specific Sample Analysis:

In-flight: Mounting and visual inspection of developing eggs. Fixations,at specific developmental stages.

Postflight: Live data movies analysis. 3D microscopy of in-situ hybridizations and whole-mount immunostained embryos. Gels and blots run and analysis.

F. Experimental Controls:

In-flight: Compare to embryonic growth in centrifuge (1-g, 2.5-g).

Ground: 1-g normal development. Development under high g (in "slow" centrifuges, 1-20-g).
Note: Eggs are damaged at the lowest table-top centrifuge speeds.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Hardware requirements:
Computerized microscope, focus control, CCS image acquisition.

Ground: Workstation for image analysis.

Experiment Identification Code: DA-e(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Konrad Dabrowski and Dr. Feng Lin

School of Natural Resources, Ohio State University
210 Kottman Hall, 2021 Coffey Road
Columbus, OH 43210

614-292-4555

Critical Question(s) Addressed:

DA-3, DA-4, DB-2, DC-1, DG-4, DG-5

3. Experiment Title

Embryonic Development of Goldfish After Artificial Fertilization in the Microgravity Environment.

4. Purpose/Hypothesis

To develop appropriate procedures for artificial fertilization (dry method) and egg incubation in the 0-g gravity environment. To test whether prolonged exposure to microgravity will induce alteration of embryonic development in fish.

5. Scientific Rationale/Rationale for Microgravity

Artificial fertilization (dry method) is a common practice in fish research as well as in hatchery production. Developing appropriate procedures for artificial fertilization and egg incubation is essential for further studies in reproductive and developmental biology of fish in the space. Whether gravity is required for normal embryonic development in teleost fish is a basic question to culture fish in the space. Understanding the early phase of embryogenesis in the microgravity environment will enable us to conduct artificial manipulation of fish, such as gynogenesis and polyploidization. Embryonic development of fish is a potential model for risk assessment of radiation exposure in the space because fish gametes are sensitive to radiation and result in easily identified morphological changes.

6. Approach

A. Experimental Duration: 6 days.

B. General Approach

Description:

Devices and supplies for handling fish out of water (e.g. hand stripping of fish for gametes) in the 0-g condition are to be worked out. For the study of embryonic development, a confocal microscopy will be used to continuously monitor the early phase of embryogenesis. Embryos will be preserved periodically for detailed histological and cytological studies of embryogenesis.

C. Number and Type of Specimen:

Fish will be induced to spawn in the space by hormonal injection. Eggs and semen from 10 outbreed fish will be collected and artificially fertilized. Fertilized eggs (10 per sampling) will be fixed every 5 minutes during the first hour post-fertilization and then every hour until hatching (expected 2 days). Larvae will be fixed every four hours until completion of yolk absorption (another 2 days).

D. Measurements/Sample

Preflight: Prepare broodstock fish from an outbreed population.

In-flight: Inject human chorionic gonadotropin for induction of gamete production. Incubate eggs in an egg incubator & fix samples according to the schedule. Stain some of the eggs with fluorescent dyes for DNA & cytoskeleton & then monitor their development with a confocal microscopy with an image analysis system attached.

Postflight: Analyze the images of the early phase of embryonic development. Section and stain the samples preserved in-flight for histological & cytological examination.

E. Specific Sample Analysis:

In-flight: Incubate eggs in a egg and fix samples (10 embryos each time). Stain some of the eggs with fluorescent dyes for DNA and cytoskeleton and then monitor their development with confocal microscopy with an image analysis system attached.

Postflight: Analyze the images of the early phase of embryonic development. Section and stain the samples preserved in-flight for histological & cytological examination.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Parallel 1-g controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Both 0-g and 1-g fish culture facilities for both adult fish and eggs; supplies for handling fish out of water (e.g. for gametes collection); confocal microscopy with an image analysis system attached.

Ground:

Experiment Identification Code: DA-f(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Konrad Dabrowski and Dr. Andrzej Ciereszko

School of Natural Resources,
The Ohio State University
2021 Coffey Road
Columbus, OH 43210

Critical Question(s) Addressed:

DA-3, DA-4, DG-4

3. Experiment Title

Effect of Microgravity on Fish Sperm Motility Characteristics and Fertilizing Ability.

4. Purpose/Hypothesis

Since movement is related to gravity, the character of sperm movement will be changed in microgravity. We plan to describe these changes by high-speed (200 frames per second) video recording and subsequent computer-aided analysis of various parameters of movement of individual spermatozoa. We will evaluate microgravity effect on sperm fertilization success. We will also test if duration of microgravity exposure will affect sperm movement characteristics in normal gravity.

5. Scientific Rationale/Rationale for Microgravity

Long-term goal of this project is to study fish reproduction in microgravity or after microgravity experience for further possible use of fishes in space missions.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

3 year old rainbow trout will be used. The advantage of using this species is seasonal spermatogenesis which enables to obtain large number (enough for paired comparisons) of mature spermatozoa over the period of 3 mo. Semen samples will be used for testing of effect of microgravity on sperm motility characteristics of fresh semen and two-week stored in 1° C in microgravity. Sperm fertilizing ability will be tested in normal gravity. Additionally, we will evaluate fertilizing potential of cryopreserved sperm stored for 90 days in microgravity.

C. Number and Type of Specimen:

10 samples of fresh semen and 20 samples of cryopreserved semen.

D. Measurements/Sample

Preflight: Collection of semen, computer-assisted sperm motility analysis, cryopreservation (may be done well in advance) and evaluation of sperm fertilizing ability.

In-flight: Computer-assisted sperm motility analysis.

Postflight: Sperm motility analysis, evaluation of sperm fertilizing ability.

E. Specific Sample Analysis:

In-flight: None

Postflight: Evaluation of videotapes.

F. Experimental Controls:

In-flight: All experiments will be performed in paired design, i.e. control samples from the same males will be held in normal gravity and analyzed at the same time like samples in microgravity.

Ground: None

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Computer-assisted sperm-motion analysis system, small refrigerator and hatching apparatus.

Ground:

Experiment Identification Code: DA-g(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Perry B. Hackett

Dept. of Genetics and Cell Biology
University of Minnesota, 1445 Gortner Ave.
St. Paul, MN 55108-1095

Critical Question(s) Addressed:

DA-2, DG-4, DA-7, DB-4

612-6246736 Fax: 612-625-5754

3. Experiment Title

Analysis of Microgravity-induced Cellular Stress in Developing Zebrafish Embryos.

4. Purpose/Hypothesis

Microgravity induces expression of cellular stress genes and/or movement of mobile elements in the zebrafish genome.

5. Scientific Rationale/Rationale for Microgravity

Cellular stress proteins, also known as heat shock proteins (HSPs), are induced by heat as well as other stressful agents, such as anoxia, alcohol, and heavy metals. In contrast to these agents, microgravity can not be experienced on earth. This raises the question of whether cells of developing embryos have the capacity to "sense" microgravity, which is stressful to animals. HSPs appear to regulate protein conformation and interactions and thus their expression is important for correct cell function. This proposal will examine not only the induction of the proteins themselves, but the activation of their promoters (genetic switches that determine the degree to which a gene has been turned on). In addition, other "sentinel" sequences, repetitive mobile elements, will be examined for the effects of microgravity on their stability. Cellular stress can lead to their mobilization, which can be detected by molecular analyses of genomic DNA.

6. Approach

A. Experimental Duration: 90 days / experiment.

B. General Approach

Description:

Experiment 1 - Induction of HSP gene expression. Zebrafish embryos, obtained from matings done in space, will be collected at several stages of development (midblastula, gastrula, neurula, etc.) and both proteins and mRNA will be isolated for analysis for HSP mRNA and Protein.

Experiment 2 - Activation of HSP promoters. Embryos from transgenic fish containing either a green fluorescence protein (GFP) gene or the B-galactosidase (lacZ) gene behind the human heat shock 70 gene promoter will be collected as described in Expt. 1 and analyzed for expression of either the GFP or lacZ gene.

Experiment 3 - Mobilization of Repetitive Elements. Genomes of gametes from zebrafish reared in microgravity will be collected for analysis of stability of repetitive elements. This experiment will test effects of microgravity on the stability of gamete genomes.

C. Number and Type of Specimen:

Several hundred embryos will be examined for experiments 1 and 2, 20-50 fish will be used to produce gametes for experiment 3.

D. Measurements/Sample

Preflight: Experiments 2 and 3: Development of hsp/GFP and/or hsp/B-gal transgenic fish and determination of appropriate primer pairs for inter-PCR screening of mobile elements.

In-flight: Expt 1: Mating of designated pairs of zebrafish & collection embryos over a 2 day period. Embryos will be collected by quick freezing in liquid nitrogen until analysis on earth. Expt 2: Embryos will be stained for lacZ activity or by fluorescence microscopy for GFP gene expression. Expt 3: Zebrafish will be mated on return to ground.

Postflight: Expt 1: RNase protection and/or RT-PCR will be used to quantify HSP RNA levels and western blots will be used to quantify HSP synthesis. Expt 2: Quantitative analysis will be done. Expt 3: Gametes from the "space fish" will be collected, their DNAs isolated, and inter-PCR analysis conducted to determine whether any of the members of different repetitive element families moved.

E. Specific Sample Analysis:

In-flight: Expt 1: light microscopy to determine that embryonic development is normal, and collecting samples of about 50-100 staged embryos in liquid nitrogen. Expt 2: Staining of zebrafish embryos and/or fluorescence microscopy at designated intervals,

Postflight: & taking pictures of the images. Expt 3: Visual examination of normal fish to ensure health.

Postflight: Expt 1: Standard RNA & protein analysis using agarose and polyacrylamide gels. Expt 2: Standard statistical analysis. Expt 3: Examination of inter-PCR products, displayed on gels, synthesized from isolated gamete DNA, & comparison with wt (pre-flight) genomes.

F. Experimental Controls:

In-flight: In all cases the results will be compared with sibling fish reared and maintained on the ground. In addition, for experiments 1 and 2, the 1x g centrifuge will allow for in-flight controls of 1xg.

Ground:

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: For Experiment 1 and 2, zebrafish mating chamber and a light "Drosophila" dissecting scope to monitor embryo development. For experiment 2, a phase contrast or DIC scope with fluorescence attachments for analysis of GFP and B-galactosidase. Experiment 3, zebrafish aquarium.

Ground: Standard molecular biology lab and for experiment 3, zebrafish rearing facility. We have all of the needed resources for this part.

Experiment Identification Code: DA-h(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Eberhard Horn

University of Ulm
Dept. of Neurology, section of Neurophysiology
Albert-Einstein-Allee 11
D - 89081 Ulm, Germany
xx49-731-502-4565 Fax: xx49-7344-3630

Critical Question(s) Addressed:

DA-2, DA-3, DA-7, DB-1, DB-2, DB-4,
DC-4, DG-2, DG-3

3. Experiment Title

Analysis of Structural and Functional Modifications of a Cricket's Gravity Sensory System During Microgravity Exposure - Genetic versus Environmental Cues.

4. Purpose/Hypothesis

One generation of crickets will be kept in space to study whether structure and function of their gravity sense organs, as well as the efficiency of the network underlying gravity induced behavior alters during ug-exposure. It is suggested that the loss of adequate sensory stimulation alters the physiological efficiency of this system because of the lack of neurotrophic effects of stimulus induced activation. Structural modifications, however, are expected to be little or absent because genetic mechanisms are dominant.

5. Scientific Rationale/Rationale for Microgravity

Long-lasting sensory deprivation can lead to irreversible changes of sensory functions. If genetic mechanisms are dominant over environmental cues, the functional efficiency of a sensory system may change within a generation, but the off-spring of deprived parents will develop normal abilities under normal environmental conditions. While these postulations can easily be tested in earth-bound experiments for the sense of vision, touch or hearing, the long-term effects of gravity deprivation can only be studied in experiments using the microgravity environment. Crickets offer a suitable model because (1) their generation cycle lasts about 70 days, (2) the development of their gravity sense organs as well as their gravity induced behavior proceeds in a steplike manner, and (3) the efficiency of their gravity sensory system can be recorded by two clear-cut approaches, the compensatory head movements during passive roll and the gravitaxic response.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Adults of the house cricket will be kept under ug-and 1g-condition until the time when the females lay their eggs. Their off-springs will be used to study, in-flight, for each instar the structural development of the peripheral sense organ as well as that of the gravity induced behavior. The consecutive generation will be reared on earth; it will be exposed to the same experimental procedures as the in-flight animals.

C. Number and Type of Specimen:

50 male and 50 female adults of the house cricket (*Acheta domesticus*) will be exposed to ug-environment, the same number to in-flight 1g-condition. 30 of them will be used as parents for the production of the consecutive generation, the other 20 for the first experiments and fixation procedures.

D. Measurements/Sample

Preflight: Investigations with a cricket generation from a limited number of lines to establish its base line development of gravity induced behavior & anatomical structures. Packing of adults from this generation into the insect holding facility before launching.

In-flight: Rearing up to the adult stage of both, the ug-exposed larvae & their in-flight 1g-control. 10 animals of each developmental stage, the gravity induced compensatory head movements will be tested under 1g-condition. Thereafter, defined afferents will be stained intracellularly by the backward-filling method in a refrigerator w/subsequent fixation. Additional fixation of 20 specimens from each stage for anatomical & electron- microscopic studies. Stowage in a freezer.

Postflight: Rearing of the next generation under earth 1g-condition & performing the same test procedures w/ each developmental stage up to the adults.

E. Specific Sample Analysis:

In-flight: None, because only recordings and fixation activities are necessary.

Postflight: Analysis of the pre-, in-flight and postflight recordings of the gravity induced head movements. Histological analyses with special reference to the development of the peripheral sensory structures and their projections into the cricket's central nervous system.

F. Experimental Controls:

In-flight: Half of the samples for 1g control: same rearing, recording and fixation procedures as the ug-exposed samples.

Ground: Same procedure as in space with 2 hrs time delay.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Specific device to record in-flight the compensatory head movements of the larval and adult crickets during 1g-exposure. This apparatus has to be developed. Refrigerator and freezer for stowage of the fixed samples. Insect holding facility with 1g-reference centrifuge and light from above.

Ground:

Experiment Identification Code: DA-i(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. John J. Brown

Department of Entomology
Washington State University
Pullman, WA 99164-6382

Critical Question(s) Addressed:

DA-2, DA-5, DA-7, DB-2, DB-3, DB-4,
DC-1, DG-3, DA-1

3. Experiment Title

Effects of Microgravity on Parasite Development Inside a Diapausing Codling Moth.

4. Purpose/Hypothesis

To determine if the absence of gravity influences the development of internal parasites.

5. Scientific Rationale/Rationale for Microgravity

Most space travelers are screened for pathogens and parasites prior to lift off, but what would happen to the development of a dormant parasite, inside the body of its host, if both host and parasite were exposed to microgravity? Would the parasite remain dormant and continued to pose no threat to the space traveler?

6. Approach

A. Experimental Duration: <90 days.

B. General Approach

Description:

Codling moth larvae exposed to Ascogaster quadridentata as eggs will be reared under short day conditions to induce diapause. Identical groups of parasitized larvae will be maintained at 0-g & 1-g during the duration of the microgravity exposure. Upon termination of the flight, both groups will be dissected and development of all parasite larvae will be examined.

C. Number and Type of Specimen:

50 parasitized codling moth larvae which would weigh 20mg each and occupy 0.1 x 10mm each.

D. Measurements/Sample

Preflight: Placement of larvae into sterilized corrugated cardboard so that each can spin a cocoon.

In-flight: No crew interaction necessary.

Postflight: Dissection of each host larvae from both populations as soon as possible.

E. Specific Sample Analysis:

In-flight: None

Postflight: Light microscopic evaluation of growth and development of each larval parasite.

F. Experimental Controls:

In-flight: 1-g described above.

Ground: Another 50 parasitized codling moth larvae maintained at the same photoperiod and temperature as the test population was exposed to during their flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Temperature should be maintained between -20 to 10 degrees C. Total weight of diapausing hosts, parasites and cardboard cocooning space would be less than 5 grams.

Ground: All Ground support would be handled by Washington State University.

Experiment Identification Code: DA-j(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Fredric J. Janzen II

Department of Zoology and Genetics
Iowa State University
Ames, IA 50011

Critical Question(s) Addressed:

DA-2, DG-1

3. Experiment Title

Can Embryonic Reptiles Develop in the Absence of Gravity?

4. Purpose/Hypothesis

The absence of gravity will not permit the embryos to attach and form an airspace. Thus, they will not survive.

5. Scientific Rationale/Rationale for Microgravity

Avian eggs possess chalazae which maintain the position of the yolk and embryo in the eggs. Reptiles do not possess these structures, hence the embryo is free floating. However, without gravity to direct the subsequent formation of the airspace (usually above the embryo), the embryo may not survive. Thus, results of such a study will lend insight into the critical function of microgravity in embryonic development.

6. Approach

A. Experimental Duration: 60-90 days, depending on the incubation temperature.

B. General Approach

Description:

Fertile eggs of red-eared slider turtles will be incubated in-flight. Four eggs will be chosen at random and will be preserved every five days or until (if?) no embryonic development is evident. The extent of embryonic development will be assessed post-flight.

C. Number and Type of Specimen:

48-64 fertilized turtle eggs.

D. Measurements/Sample

Preflight: Fertilized turtle eggs will be obtained from a commercial turtle farm and placed on the shuttle the day of the flight.

In-flight: Incubation temperature will be maintained constant. Water will be added once weekly to keep the incubation substrate moist.

Postflight: Analysis of the extent of embryonic development. This will involve microscopic examination of preserved embryos.

E. Specific Sample Analysis:

In-flight: Regular preservation of eggs/embryos.

Postflight: Comparison of rates and extent of embryonic development to those of Earth-incubated eggs.

F. Experimental Controls:

In-flight: None

Ground: Normal laboratory controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Dent fixative, individual jars for the preserved eggs/embryos.

Ground: Clearing and staining solutions, microscope.

Experiment Identification Code: DA-k(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Bernard C. Wentworth

Department of Poultry Science
University of Wisconsin
Madison, WI 53706-1284

Critical Question(s) Addressed:

DA-2, DA-3

3. Experiment Title

Effect of Weightlessness on Extra Embryonic Migration in Birds.

4. Purpose/Hypothesis

This experiment will test the hypothesis that a gravitational field is required for normal migration of extraembryonic membranes of the quail embryo.

5. Scientific Rationale/Rationale for Microgravity

There is substantial documentation that the avian embryo develops the best if it is turned frequently early in embryonic development. Also normal development is improved if eggs are incubated with the large end up. Embryonic hematopoiesis, primary germ cell migration, metabolism, respiration, and calcium absorption are all dependent on patent extra embryonic membranes.

6. Approach

A. Experimental Duration: 2 - 16 days.

B. General Approach

Description:

Fertile eggs will be incubated in flight. There would be reference controls represented by synchronous and laboratory control.

C. Number and Type of Specimen:

Replicated flights of 48 incubated fertile quail eggs each would be ideal. One-half (24) in each replication would be in a 1g centrifuge would be incubated in microgravity.

D. Measurements/Sample

Preflight: Flock producing eggs should be managed to ensure nearly 100 percent fertility.

In-flight: Both groups of incubating eggs, 1g and microgravity, should be turned at least three times each day. Three eggs from each group should be fixed in 4 percent paraformaldehyde on days 2, 4, 6, 8, 10, 12, 14, and 16 after the start of incubation. The reference ground control will be handled the same as the in-flight group.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Fixed samples from days 2, 4, and 6 will be sectioned and subjected to the appropriate histological stain and DNA probes to identify normality of extra embryonic membrane migration. Sub samples of the extra embryonic membranes and embryos will be taken on days 8, 10, 12, 14 and 16. The same biological probes and histological stain will also be applied to the older samples.

F. Experimental Controls:

In-flight: None

Ground: None

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

Experiment Identification Code: DA-I(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Prof. J. Whitfield Gibbons

Savannah River Ecology Laboratory
University of Georgia
P.O. Drawer E
Aiken, SC 29802

Critical Question(s) Addressed:

DA-2, DG-3

3. Experiment Title

Effects of Weightlessness on Embryonic Positioning of Reptile Embryos.

4. Purpose/Hypothesis

The experiment will test the hypothesis that a gravitational field is necessary for embryo orientation in early developmental stages of reptile eggs.

5. Scientific Rationale/Rationale for Microgravity

Reptile eggs vary in embryonic positioning during early development with the fertilized ovum in turtles migrating to an upper position within the albumen portion of the egg whereas the fertilized ovum of oviparous snakes and lizards remains stationary. Little is known of the mechanism for embryonic movement in turtles nor of the disparity between turtles and squamates. Testing early embryonic responses in a non-gravity situation could reveal developmental features heretofore undiscovered and possibly provide explanations for the divergent ecological and evolutionary features between the two orders.

6. Approach

A. Experimental Duration: 30-90 days.

B. General Approach

Description:

Eggs of freshwater turtles (*Trachemys scripta*, *Kinosternon subrubrum*) and terrestrial snakes (*Elaphe guttata*, *Lampropeltis getulus*) will be exposed to normal and 0 gravitational fields within 24 hours of oviposition (developmental delay can be induced through maintaining recently deposited eggs in a simulated oviductal environment that inhibits development). Eggs will be frozen at different stages (24-, 48-, 72-hour, 6-day, 12-day, and final hatching) under both treatments.

C. Number and Type of Specimen:

For adequate statistical testing, a total of 144 eggs will need to be used for each treatment. The number can be reduced to half by eliminating two of the species.

D. Measurements/Sample

Preflight: Snake and turtle eggs will be obtained by forced ovulation using oxytocin and then kept in a simulated oviductal state until initiation of the experiment.

In-flight: Provision will have to be made to freeze selected eggs during the non-gravitational period; similar measures will be taken with the control eggs kept in the laboratory.

Postflight: Eggs from both treatments will be maintained in frozen state until dissections and analyses are made.

E. Specific Sample Analysis:

In-flight: None

Postflight: Frozen eggs will be dissected to determine embryo positions and lipid and protein analyses will be performed to determine differential use of embryos under various gravity treatments

F. Experimental Controls:

In-flight: (pre-flight) Lipid and protein analyses will be performed on newly oviposited eggs and on those kept in simulated oviductal state for comparison with ground-based controls and weightlessness treatment eggs.

Ground: Ground-based laboratory incubation procedures to correspond with timing of weightlessness treatments.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Reptile egg incubators

Ground: Oxytocin supply
X-ray equipment
Holding cages for snakes

Experiment Identification Code: DA-m(2) D = developmental; A = early developmental events

1. Discipline: Developmental Biology

2. Author(s): Prof. Hefzibah Eyal-Giladi

Department of Cell and Animal Biology
Hebrew University
Jerusalem 91904
Israel

Critical Question(s) Addressed:

DA-2, DA-6, DG-1

3. Experiment Title

Is Development of Avian Embryonic Axis Gravity Dependent?

4. Purpose/Hypothesis

Under microgravity conditions the initially radially symmetrical avian blastodisc will lack the instruction (by gravity) to change into a bilateral system, which is required for the formation of a bilateral symmetric embryo.

5. Scientific Rationale/Rationale for Microgravity

Ground observations have shown that during the prelaying uterine period, the avian blastoderm is in a tilted position and that the uppermost side will always develop into the posterior side of the future embryo. Manipulations both in vivo and in vitro have shown that during the sensitive period (14-16 h of the eggs' sojourn in the uterus) one can change the direction of the future axis by changing the spatial position of the blastodisc. In experiment in which the blastodisc has been forced into a horizontal position, no embryonic axis developed and the growing blastoderm has retained its radial symmetry.

6. Approach

A. Experimental Duration: Four days.

B. General Approach

Description:

Shell-less uterine quail eggs will be aborted (10 h uterine age) and immediately put into previously prepared empty shells. The remaining empty space in the shell will be filled up with an adequate physiologic solution to compensate for the lack of normal plumping of uterine fluids. The shells will be sealed and the reconstructed egg refrigerated until and through launching. In flight the eggs will be incubated for 20 h at 41°C in an atmosphere containing 8-10% CO₂ and then transferred to a 37°C incubation.

C. Number and Type of Specimen:

30 aborted and reconstructed quail eggs.
10 normally laid quail eggs.

D. Measurements/Sample

Preflight: Preparation of aborted eggs.

In-flight: (1) Fixation of 5 experimental eggs and 5 centrifuge controls after the first 41°C incubation period.
(2) Fixation of 5 eggs from each group as well as the normally laid egg after additional 24 h and 72 h

Postflight: Examination of fixed material.

E. Specific Sample Analysis:

In-flight: None

Postflight: Examination of fixed material.

F. Experimental Controls:

In-flight: (1) If available, aborted eggs in 1-g centrifuge.
(2) Normally laid eggs.

Ground: Controls will parallel flight experiments.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Adequate incubators.

Ground:

Experiment Identification Code: DB-a(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Emily Morey-Holton and Dr. Alexandre Malouvier

NASA Ames Research Center
mail stop 236-7
Moffett Field, CA 94035-1000

Critical Question(s) Addressed:

DB-1, DB-3

phone: (415) 604-5471 fax: (415) 604-3159

3. Experiment Title

Rat Skeletal Adaptation to Spaceflight and Readaptation to 1g.

4. Purpose/Hypothesis

The purpose of this experiment is to investigate if the reported spaceflight-induced osteopenia is only dependent on mechanical usage and if it can be prevented by inflight centrifugation.

5. Scientific Rationale/Rationale for Microgravity

Spaceflight induced microgravity provides a unique tool to unload the skeleton. For the first time we will have reliable spaceflight -1g controls as well as extended flight duration that will allow us to discriminate the effects of spaceflight from those of microgravity. We will also be able to observe the effects of recovery on loads applied to bones.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

First, obtain baseline values on strain applied to two weight-bearing bones with differing weight-bearing characteristics (tibia and humerus) as well as blood concentrations of pH, calcium and phosphorous. Then observe how microgravity and suspension affect these parameters during 90 days. Finally the effects of 90 day recovery at 1g will be observed.

C. Number and Type of Specimen:

36 Sprague-Dawley male rats (12 flight; 6 microgravity, 6 centrifuge. 24 ground, 6 baseline controls, 6 vivarium controls, 6 ground controls, 6 tail suspended).

D. Measurements/Sample

Preflight: Implantation of strain gauges and biochemistry probes. Baseline data (strain, ionized blood calcium). Double labeling with calcein and tetracycline. Implantation of minipumps for bone labeling if no injection possible in-flight.

In-flight: Record flight data (stain, ionized blood calcium). If possible, inject rats with bone labels.

Postflight: Recovery data (stain, ionized blood calcium). Double labeling with calcein and tetracycline. Sacrifice and dissection.

E. Specific Sample Analysis:

In-flight: Collect both telemetry data and video data and downlink at one week intervals during the flight.

Postflight: Same as inflight.

F. Experimental Controls:

In-flight: 1g-onflight Controls on the centrifuge (n = 6, FC).
Microgravity-Flight (n = 6 F).

Ground: Baseline controls (BC), Vivarium Controls (VC), 1g Controls (GC), Suspended (S).

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

Experiment Identification Code: DB-b(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Thomas Coffman, M.D. and Christopher Best

Nephrology Research
Duke University Medical Center

Critical Question(s) Addressed:

DB-1, DB-4

3. Experiment Title

The Role of AT_{1A} Receptors for Angiotensin II in Physiological Responses to Alterations in Gravity.

4. Purpose/Hypothesis

Determine the effects of microgravity on biological responses in mice with specific alterations in genes in the renin angiotensin system.

5. Scientific Rationale/Rationale for Microgravity

The renin angiotensin system is one of the critical regulators of blood pressure and fluid homeostasis and is involved in adaptive responses to environmental conditions which alter pressure and fluid balance. It would be of both biological and practical interest to examine responses to altered G-forces in our mutant renin angiotensin system.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

We will compare responses in mice that have targeted disruption of the gene encoding the AT_{1A} receptor for angiotensin II. Animals that are homozygous for this mutation lack detectable AT₁-specific binding in kidney and have reduced levels of resting blood pressure. We will compare responses in homozygous knockout mice (Agt^r 1A(-/-)) with wild type controls. Biologic responses of interest would include: Blood pressure, heart rate, blood flow, deep body temperature. These would be monitored using biotelemetry sensors.

C. Number and Type of Specimen:

6 +/+ male mice	= At .1 microgravity.	6 +/+	= on the 2.5 meter centrifuge.
6 -/- male mice		6 -/-	

D. Measurements/Sample

Preflight: Genotyped animals with 1 week BP/heart rate measurements following implantation prior to flight.

In-flight: Biotelemetry data, weekly body weights, food and H₂O intake for the duration of flight.

Postflight: Collection of blood, kidneys, adrenal glands, heart and brain in fixative, N₂ frozen and samples for immunopathology.

E. Specific Sample Analysis:

In-flight: None

Postflight: Hormone assays of blood. Histology and autoradiography of tissues.

F. Experimental Controls:

In-flight: 6 +/+
= Mice in 2.5 meter centrifuge.

6 -/-
Ground: 6 +/+
= Mice in a habitat similar to that of flight.
6 -/-

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Glovebox, mass measurement device.

Ground:

Experiment Identification Code: DB-c(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Tuan Vo-Dinh

Advanced Monitoring Development Group
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6101
423-574-6249 Fax: 423-576-7651

Critical Question(s) Addressed:

DB-1

3. Experiment Title

Monitoring in vivo Calcium Levels in Adult Rats in Microgravity.

4. Purpose/Hypothesis

To determine the effects of microgravity on calcium production in the body.

5. Scientific Rationale/Rationale for Microgravity

The main objective of this research project is to develop in vivo sensors to determine on a real-time or near real-time basis the effects of microgravity in the production levels of calcium in the body. Calcium is an important biological constituent that has been found to be affected by long periods under microgravity. The proposed experiments with the in vivo calcium sensors could lead to a better understanding of the bone demineralization resulting from exposure to space flight conditions.

6. Approach

A. Experimental Duration: 10-60 days.

B. General Approach

Description:

Animals will be implanted (or instrumented) with calcium sensors equipped with biotelemetry devices before flights. The levels of calcium will be monitored continuously during flights and analyzed in conjunction with other physiological parameters.

C. Number and Type of Specimen:

6 adult male rats, 6 adult female rats at microgravity.
6 adult male rats, 6 adult female rats on the centrifuge.

D. Measurements/Sample

Preflight: Selection of flight animals determined by display of normal physiological conditions following implantation of sensors prior to launch.

In-flight: Calcium determination performed on real-time basis.

Postflight: All animals will be returned for collection of blood and tissue samples.

E. Specific Sample Analysis:

In-flight: None

Postflight: Assays of calcium on blood and tissues.

F. Experimental Controls:

In-flight: 24 animals.

Ground: 12 control animals in an habitat similar to that of flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: None

Ground:

Experiment Identification Code: DB-d(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Klaas Kramer Aalt Bast, PhD

Vrije Universiteit
Dept. of Pharmacochimistry,
de Boelelaan 1083 1081 HV Amsterdam,
The Netherlands
31 20 4447581 Fax: 31 20 4447610

Critical Question(s) Addressed:

DB-1

3. Experiment Title

Anti-oxidant Status in Freely Moving Mice in Microgravity.

4. Purpose/Hypothesis

To determine the effects of microgravity on anti-oxidant status. The oxidant stress will be imposed on the animal by a well-described doxorubicin-induced cardiotoxicity in mice.

5. Scientific Rationale/Rationale for Microgravity

Question: Will microgravity influence the ability of the organism to cope with oxidative stress.
Experiment: The cardiotoxicity of the cytostatic agent doxorubicin is caused by the formation of oxygen free radicals. The anti-oxidant levels will be reflected in the time course of the doxorubicin induced cardiotoxicity. Results: These experiments will indicate if microgravity changes the antio-oxidant levels. Conclusion: A changed oxidant/anti-oxidant balance during microgravity would be of importance for the diet of the astronaut.

6. Approach

A. Experimental Duration: 56 days.

B. General Approach

Description:

Animals will be group housed and instrumented with biotelemetry sensors for temperature, ECG and heart rate.

C. Number and Type of Specimen:

12 male Balb/c mice at microgravity and 12 male Balb/c mice on the 2.5 meter centrifuge.

D. Measurements/Sample

Preflight: Selection of flight animals (6 mice with doxorubicin-induced cardiotoxicity and 6 mice without per gravity condition) determined by display of ECG following implantation of sensor prior to launch.

In-flight: Biotelemetry data, weekly body weights, food and water intake. All animals in microgravity and all animals from the centrifuge will be killed for blood (separation of erythrocytes/plasma) and tissue collection after 56 days. 3 hearts will be preserved in fixative & 3 hearts, lung & liver quickly frozen from each group.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Assays of anti-oxidant levels in erythrocytes/plasma and in the other tissues. Heart: weight/histology.

F. Experimental Controls:

In-flight: 12 animals on the 2.5 meter centrifuge (6 animals with and 6 animals without doxorubicin-induced cardiotoxicity).

Ground: 12 animals (6 animals with and 6 animals without doxorubicin-induced cardiotoxicity) in a habitat similar to that flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Glovebox, mass measurement device, dissection equipment, quick/snap freezer, fixation equipment.

Ground:

Experiment Identification Code: DB-e(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): SSBRP - Space Station Research Project Biological

NASA - Ames Research Center
c/o Caye Johnson
m/s 244-19
Moffett Field, CA 94035

Critical Question(s) Addressed:

DB-1

3. Experiment Title

Effect of Microgravity on Regional Blood Flow and Distribution.

4. Purpose/Hypothesis

Changes in blood flow and distribution produce alterations in microvascular structure and function.

5. Scientific Rationale/Rationale for Microgravity

Blood flow in particular tissues may be dependent upon gravity for proper function. This experiment will determine the influence of gravity (or lack thereof) on vascular structure and function in particular tissues.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Specimens will be instrumented with right atrial and aortic pressure catheters, an aortic electromagnetic flow probe, and a dorsal microcirculatory chamber. All instrumentation and the dorsal microcirculatory chambers will be surgically installed preflight.

C. Number and Type of Specimen:

12 male Sprague Dawley rats starting at 175-grams (6 on centrifuge / 6 on holding rack).

D. Measurements/Sample

Preflight: Measurements of heart rate, right atrial pressure and aortic pressure will be measured weekly. In addition, microcircular chambers will be photographed and still and video images collected in a weekly basis.

In-flight: Measurements will be conducted weekly, as for preflight , and specimen mass will be determined every other week. Drug studies may be conducted using adrenergic agonists and antagonists to examine changes in reflex control and sensitivity.

Postflight: All specimens will be returned following the 90 day increment and measurements taken weekly until responses return to preflight levels.

E. Specific Sample Analysis:

In-flight: See above.

Postflight: See above.

F. Experimental Controls:

In-flight: 6 on centrifuge.

Ground: 12 rats on the ground for duration of experiment.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Glovebox, mass measurement device and camera.

Ground:

Experiment Identification Code: DB-f(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): SSBRP - Space Station Biological Research Project

NASA - Ames Research Center
c/o Caye Johnson
m/s 244-19
Moffett Field, CA 94035

Critical Question(s) Addressed:

DB-1, DB-3

3. Experiment Title

Effect of Microgravity on the Ultrastructure of the Rat Neuromuscular Junction.

4. Purpose/Hypothesis

Ultrastructural analysis of skeletal muscle removed in flight will characterize the changes in myofibrils, mitochondrial morphology and distribution, and neuromuscular junction anatomy resulting from long-term exposure to microgravity.

5. Scientific Rationale/Rationale for Microgravity

Ultrastructural features of selected rodent muscles may be particularly sensitive to the effects of gravity or lack thereof. This experiment will determine the long term effects of microgravity on muscular cytoskeleton morphology.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Specimens will be housed both in the Habitat Holding Unit (0g) and on the Centrifuge at 1g. Each specimen will be implanted with EMG telemetry which will be monitored at regular intervals.

C. Number and Type of Specimen:

24 male Sprague Dawley rats; 16 on holding rack at microgravity; 8 on centrifuge at 1-g.

D. Measurements/Sample

Preflight: Measurement of EMG to assure recovery from surgery & proper function of implant.

In-flight: At 2, 4, 8 & 12 weeks, 4 specimens housed at 0-g will be perfused, muscles removed and fixed for later analysis. At 4 & 12 weeks, 4 specimens from the centrifuge control group will be perfused, muscles removed and fixed for ground-based electron microscopic analysis.

Postflight: Electron microscopy at P.I.'s lab.

E. Specific Sample Analysis:

In-flight: None

Postflight: Electron microscopy at P.I.'s lab.

F. Experimental Controls:

In-flight: Centrifuge.

Ground: None

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Glovebox, surgical supplies

Ground:

Experiment Identification Code: DB-g(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Prof. Jacob S. Ishay

Dept. of Physiology & Pharmacology
Sackler Faculty of Medicine
Tel-Aviv University
Israel

Critical Question(s) Addressed:

DA-2, DB-1, DB-2, DB-4, DG-2

3. Experiment Title

Hornet Comb Building Behavior in Space.

4. Purpose/Hypothesis

To determine the effect of n-g and food supplements on comb building behavior in juvenile and adult worker hornets.

5. Scientific Rationale/Rationale for Microgravity

On earth, hornets are dependent on gravity as a cue to orient and size their combs. Several sensory modalities are believed to be involved and a developmental component is also involved. In ground based centrifugation/orientation studies, juvenile workers are influenced by a resultant gravity vector, but adult workers still derive cues from earth's gravity.

6. Approach

A. Experimental Duration: 21 days.

B. General Approach

Description:

8 groups of 10 hornets each will be sent into space. 4 groups of juveniles, 4 groups of adults. 2 groups from developmental stage will be housed at 0-g and 2 groups will be housed at 1-g. Comb building behavior will be examined. Water will be supplemented with 5% ethanol as a productivity stimulant in 4 of 8 groups.

C. Number and Type of Specimen:

80 hornets (*Vespa orientalis*), 40 adults (1-3 days of age) and 40 juveniles (0-24 hrs) will be used from an known line of hornets.

D. Measurements/Sample

Preflight: Solution of offspring.

In-flight: Comb building behavior, productivity/activity and feeding.

Postflight: None

E. Specific Sample Analysis:

In-flight: Examine comb building behavior, productivity/activity, and egg laying.

Postflight: Continuous observation of comb building, egg laying, behavior (morphometric examination of comb cells).

F. Experimental Controls:

In-flight: See General Approach above.

Ground: None

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Specimen chambers for comb building. Controlled environment and low light. Food and water supplements.

Ground:

Experiment Identification Code: DB-h(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Frieda B. Taub

School of Fisheries
University of Washington
Box 355100
Seattle, WA 98195

Critical Question(s) Addressed:

DB-2, DC-2, DC-3, DC-4, DG-2, DG-4,
DE-3

3. Experiment Title

Embryological Development and Early Larval Development of Medaka.

4. Purpose/Hypothesis

Medaka, a small fish, has shown sensitivity to chemical factors: it can be expected to show responses to weightlessness, especially in development of the inner ear.

5. Scientific Rationale/Rationale for Microgravity

We do not know if inner ear development and balance control depend on a consistent orientation to gravity.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Culture eggs and larvae of Medaka under conditions of weightlessness. Medaka adults have been shown to tolerate weightlessness and produce eggs; however they have cannibalized them prior to study. We have proposed some devices that make it feasible to strip the eggs and save them from hungry parents. Medaka embryology is well characterized. Eggs should be preserved at various stages, and some young allowed to survive and grow to adulthood.

C. Number and Type of Specimen:

100 Medaka eggs

D. Measurements/Sample

Preflight: Feeding and cleaning.

In-flight: Feeding.

Postflight: Recovery; preservation of some for behavior studies.

E. Specific Sample Analysis:

In-flight: Medaka development from egg to juvenile (video), swimming ability.

Postflight: Embryological development, especially of the inner ear; orientation to earth gravity, swimming ability, stamina. It has been reported that Medaka raised in gas-free systems do not fill their air bladder; this should be confirmed and it should be determined if this is a problem at 1-g, but not at weightless conditions.

F. Experimental Controls:

In-flight: Survival; swimming behavior.

Ground: Compare swimming and orientation of fish reared since under microgravity since ovulation with earth born sibs. Growth rates and certain aspects of ear development could be determined by examining the otoliths (ear bones).

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Minimum on orbit requirements.

Ground: Hardware requirements for ground.

Experiment Identification Code: DB-i(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): P.S. Steyger, Ph.D

R.S. Dow Neurological Sciences Institute,
Legacy Good Samaritan Hospital & Medical Center
1120 NW 20th Ave., Portland Oregon, 97209

Critical Question(s) Addressed:

DB-1, DB-2, DB-3, DC-4, DG-2, DG-4

503-229-7414 Fax: 503-229-7229

3. Experiment Title

Vestibular Hair Cell Regeneration in Microgravity.

4. Purpose/Hypothesis

Does microgravity affect hair cell regeneration in otolith organs following trauma?

5. Scientific Rationale/Rationale for Microgravity

The amphibian inner ear induces rapid hair cell regeneration following ototoxicity in both in vivo and in vitro conditions. Amphibians represent an animal model for investigating how hair cell regeneration can be accomplished in the mammalian auditory and vestibular systems, essential sensory systems for astronauts. Therefore it is necessary to determine if microgravity alters the pattern of mitotic hair cell regeneration in the inner ear following trauma.

6. Approach

A. Experimental Duration: 10 days maximum for in vitro experiments.

B. General Approach

Description:

Amphibian vestibular otolith organs prepared for organ culture will be subjected to ototoxic insult in microgravity. Subsequently, organs will be allowed to recover for up to 9 days, prior to fixation and immunocytochemical processing for cytoskeletal proteins (actin, tubulin, cytokeratins), BrdU uptake, and anatomical analysis.

C. Number and Type of Specimen:

One utricle and saccule per organ chamber, obtained from the bullfrog, Rana Catesbeiana. Ten organ chambers per organ culture experiment.

D. Measurements/Sample

Preflight: Prepare organ cultures of vestibular otolith organs for mitotic hair cell regeneration.

In-flight: Induce ototoxic insult in organ cultures, change culture medium every 24 hours, fix organs after pre-determined period of recovery.

Postflight: Process samples for immunocytochemistry, and scanning electron microscopy.

E. Specific Sample Analysis:

In-flight: Determine organ culture viability on daily basis.

Postflight: At each recovery period determine: (1) number of immature hair cells, & correlate with BrdU-labeled cells. (2) immunocytochemical distribution of: (a) actin in regenerating hair cells, particularly the hair bundle; (b) tubulin, (c) cytokeratins, a marker of supporting cells, & expanding supporting cell apices sealing the reticular lamina following hair cell loss; (d) expression of transduction channels in regenerating hair bundles of BrdU labeled hair cells, to determine the functional maturity of regenerating hair cells. (3) ultrastructure of the reticular lamina in regenerating organs.

F. Experimental Controls:

In-flight: 1-g organ cultures to control for shake-rattle-and roll effect.

Ground: Organ cultures in identical equipment to control for effects induced by equipment.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Preflight: Organ culture chambers, ability to replace organ culture media, and subsequently fix organs, dissecting microscopes, binocular microscope with Nomarski optics, frog aquaria.

Ground: Post flight: Dissecting microscopes, fume chambers, video-enhanced computer-assisted fluorescent and Nomarski microscopes, scanning electron microscope (all available at PI's location).

Experiment Identification Code: DB-j(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): P.S. Steyger, Ph.D

R.S. Dow Neurological Sciences Institute,
Legacy Good Samaritan Hospital & Medical Center
1120 NW 20th Ave., Portland Oregon, 97209

503-229-7414 Fax: 503-229-7229

Critical Question(s) Addressed:

DB-1, DB-2, DB-3, DB-4, DC-4, DG-2,
DG-4

3. Experiment Title

Non-mitotic Hair Cell Regeneration in Microgravity.

4. Purpose/Hypothesis

Does microgravity affect non-mitotic hair cell regeneration in otolith organs following trauma?

5. Scientific Rationale/Rationale for Microgravity

The amphibian inner ear induces extremely rapid hair cell regeneration following ototoxicity in vitro, in the presence of aphidicolin, a mitotic blocker. Thus, hair cell regeneration must take place via non-mitotic mechanisms, through the repair of sub-fatally damaged hair cells, or by the direct transdifferentiation of supporting cells into hair cells, without an intervening cell division. This latter process provides a non-mitotic regenerative mechanism for traumatized auditory and vestibular systems, essential sensory systems for astronauts. Therefore, it is necessary to determine if microgravity alters the pattern of non-mitotic hair cell regeneration in the inner ear.

6. Approach

A. Experimental Duration: 10 days maximum.

B. General Approach

Description:

Amphibian vestibular otolith organs prepared for organ culture will be prevented from undergoing mitotic division by supplementing all organ media with aphidicolin, a mitotic blocker. Organs will be subjected to ototoxic insult in microgravity. Subsequently, organs will be allowed to recover for up to 9 days, prior to fixation and immunocytochemical processing for cytoskeletal proteins (actin, tubulin, cytokeratins), BrdU uptake, and anatomical analysis.

C. Number and Type of Specimen:

One utricle and saccule per organ chamber, obtained from the bullfrog, Rana catesbeiana. Ten organ chambers per experiment.

D. Measurements/Sample

Preflight: Prepare vestibular otolith organ cultures in media supplemented with aphidicolin (a mitotic blocker) for incubation in flight equipment.

In-flight: Induce ototoxic insult, replace organ culture medium (supplemented with aphidicolin) every 24 hours, fix organs after predetermined period of recovery.

Postflight: Process samples for immunocytochemistry, and scanning electron microscopy.

E. Specific Sample Analysis:

In-flight: Determine organ culture viability on daily basis.

Postflight: At each recovery period determine: (1) the number of immature hair cells, & correlate with BrdU-labeled cells. (2) Immunocyto- chemical distribution of: (a) actin in regenerating hair cells, particularly to the hair bundle; (b) tubulin (c) cytokeratins, a marker of supporting cells, & expanding supporting cell apices sealing the reticular lamina following hair cell loss; (d) expression of transduction channels in regenerating hair bundles, to determine the functional maturity of regenerating hair cells. (3) ultrastructure of the reticular lamina (epithelial surface) in regenerating organs.

F. Experimental Controls:

In-flight: 1-g organ cultures to control for shake-rattle-and roll effect.

Ground: Organ cultures in identical equipment to control for effects induced by equipment.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Preflight: Organ culture chambers, ability to replace organ culture media, and subsequently fix organs, dissecting microscopes, binocular microscope with Nomarski optics, frog aquaria.

Ground: Post flight: Dissecting microscopes, fume chambers, video-enhanced computer-assisted fluorescent and Nomarski microscopes, scanning electron microscope (all available at PI's location).

Experiment Identification Code: DB-k(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): P.S. Steyger, Ph.D

R.S. Dow Neurological Sciences Institute,
Legacy Good Samaritan Hospital & Medical Center
1120 NW 20th Ave., Portland Oregon, 97209

Critical Question(s) Addressed:

DB-1, DB-2, DC-4, DG-1, DG-4

503-229-7414 Fax: 503-229-7229

3. Experiment Title

Otoconial Development in Microgravity.

4. Purpose/Hypothesis

Does microgravity affect the ultrastructural development and morphogenesis of otoconia during embryo development in space.

5. Scientific Rationale/Rationale for Microgravity

Otoconia are essential for the detection of inertia and gravity, acting as mass-loaders upon the extra-cellular membranes overlying the mechanosensory receptor cells in the vestibular system. Previous studies have shown that hypergravity could be expected to increase the volume of otoconia/otolithic membrane. This could lead to vestibular dysfunction (dizziness/vertigo) in developing organisms. Ultrastructural observations of otoconia from the IML-2 mission had spiculated or pitted surfaces in contrast to the striated or smooth surfaces seen during terrestrial development. Therefore a rigorously controlled ultrastructural experiment is essential to further examine otoconial development in microgravity.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Amphibian eggs and larvae will be obtained from impregnated female adult newts, Cynops pyrrhogaster, using hormone treatment. Eggs will be obtained in space flight, or 1-2 day old eggs obtained at Ground Control could also be used. Eggs and larvae will be raised in both 0-g and 1-g (centrifugal) conditions during flight, and at 1-g on the ground. Larvae at periodic intervals (and hence different stages of labyrinthine development) will be fixed, dehydrated and returned to ground control for scanning electron microscopy.

C. Number and Type of Specimen:

50 eggs/larvae per cassette (already designed and used) and 2 female adult newts, Cynops pyrrhogaster, per cassette. (Alternate species: Rana catesbeiana, or other Rana Species)

D. Measurements/Sample

Preflight: Obtain impregnated females, store at 4°C, induce egg laying with human chorionic gonadotrophin hormone.

In-flight: Second hormone injection for adults, monitor larval growth. At desired growth stage, fix samples briefly (<2 hours), and dehydrate.

Postflight: Process samples for scanning electron microscopy.

E. Specific Sample Analysis:

In-flight: Obtain growth stages of larvae on daily basis.

Postflight: Ultrastructural analysis of otoconia at different developmental stages.

F. Experimental Controls:

In-flight: 1-g larvae to control for shake-rattle-and roll effect.

Ground: Larvae in identical equipment to control for growth disturbances induced by equipment.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: The AAEU rack used in IML-2 (July 1994), and the 1-g centrifuge, plus cassettes designed for these experiments, plus fixation capsules and an on-board dessicator/vacuum pump.

Ground: On the ground dissecting microscopes with fine dissecting tools, sputter coater and SEM facility will be required, and are available.

Experiment Identification Code: DB-l(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Darcy A. Reed and Dr. Nancy E. Beckage

Dept. of Entomology/5419 Boyce Hall
University of California - Riverside
Riverside, CA 92521-0314

Critical Question(s) Addressed:

DB-2, DC-1, DA-2, DG-2, DG-3

909-787-3521

3. Experiment Title

Effects of Microgravity on Cocoon Spinning and Metamorphosis of Insects.

4. Purpose/Hypothesis

To examine cocoon spinning and metamorphosis of both free-living and parasitic insects
- specifically - test for geotropic cues necessary for cocoon formation and successful metamorphosis in lepidopteran insects and their hymenopteran parasites.

5. Scientific Rationale/Rationale for Microgravity

In some lepidopteran host-parasitic wasp systems two cocoons are formed, that of the host and that of the parasite. In other systems the parasites emerge through the cuticle of a naked or non-cocoon spinning host. Successful eclosion of wasp adults depends on the completion of the cocoon. The absence of a cocoon results in premature wasp mortality. In this proposed test, the insect larvae would be exposed to microgravity conditions which may result in the formation of irregular cocoons and jeopardize the success of the emerging adults. Sensory cues normally facilitating cocoon spinning and orientation are currently unknown.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

Easily reared insects will be used at the prewandering stage and will not need to be fed during the experiment. These larvae will be reared in 0-g and 1-g conditions at ambient temperatures and pressures in the GBF until the adults emerge.

C. Number and Type of Specimen:

Tobacco hornworm larvae, parasitized by the gregarious endoparasitoid Cotesia congregata, will be used for the naked type of relationship in which only the parasitoids spin cocoons. Cabbage looper larvae parasitized by the solitary endoparasitoid, Chelonus sp., will be used as an example of host and parasitoid cocoon spinning. The Mediterranean flour moth readily spins cocoons on any substrate. 20 larvae of each type parasitized and nonparasitized will be used in each

D. Measurements/Sample

Preflight: Laboratory reared larvae which have stopped feeding will be selected for the experiment. A substrate will be provided onto which the hosts can attach within the confinement container to prevent the host from bouncing off the sides of the container.

In-flight: Observations will be made as to the spinning capabilities of the insects. The naked tobacco hornworm larvae which were parasitized will be observed as to the location of the emerging parasitoids. All insects will be observed for adult emergence.

Postflight: Emerging adults will be noted and structure on the cocoons will be observed.

E. Specific Sample Analysis:

In-flight: The location of the emerging Cotesia larvae will be monitored. In nature, these wasps emerge only from the dorsal and lateral aspects of the host and are primarily located in the posterior region. Success or failure to spin cocoons by both wasp larvae and caterpillars will be noted. The amount of time taken to spin cocoons will also be noted.

Postflight: None

F. Experimental Controls:

In-flight: Same as above only under 1-g conditions.

Ground: 20 lab reared parasitized larvae of each type.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Containers to hold the larvae in temperature and atmospheric conditions. 1 and 5 oz. plastic cups with lids or petri dishes with septa for the larvae to attach.

Ground:

Experiment Identification Code: DB-m(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Sonny Ramaswamy

Department of Entomology & Plant Pathology
Mississippi State University, MS 39762-9775

Critical Question(s) Addressed:

DA-2, DB-1, DB-2, DB-4

601-325-2085 Fax 601-324-8837

3. Experiment Title

Effect of Gravity on Wing Expansion in Newly Eclosed Tobacco Budworm Moths.

4. Purpose/Hypothesis

Moths and butterflies perch on vertical substrates immediately upon emergence and depend on gravity for expansion of their wings, which takes approximately 90-120 minutes in the tobacco budworm, *Heliothis virescens*, on earth. It is hypothesized that under zero gravity conditions of the space shuttle, the wings should not expand and, therefore, these insects will be unable to fly.

5. Scientific Rationale/Rationale for Microgravity

As noted above, adult insects upon emergence utilize gravitational forces to help in expanding soft body parts. The proposed experiment tests this hypothesis. If, in future, humans colonized space and grow food crops requiring insect-mediated pollination, insects with fully expanded wings might be a consideration.

6. Approach

A. Experimental Duration: 12-20 days.

B. General Approach

Description:

Sibling tobacco budworm pupae will be separated into two groups. One group will be held in emergence cages on the space shuttle, while the other will be held on earth. Moth emergence will be observed once every 15 minutes between 1 h and 3 h after lights off. Time of emergence for each moth will be noted. After 180 minutes, moths will be sacrificed and wing length and width will be measured using calipers.

C. Number and Type of Specimen:

One hundred laboratory reared pupae will be separated into 2 groups of fifty, one to be flown on the shuttle and the other to be maintained in the laboratory on ground.

D. Measurements/Sample

Preflight: None

In-flight: Observe moth emergence between 1 h and 3 h after lights off. Measure wing length and width at widest part at 180 minutes after emergence.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: None

F. Experimental Controls:

In-flight: None

Ground: Sibling moths will be maintained in a 14 h Light:10 h dark photoperiod, both on the shuttle and on ground. No other controls are necessary.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Collapsible steel screen cages (15 cm³) to hold moths. Plastic petri dishes (ca. 9 cm) for holding pupae. Moist paper toweling to line bottoms of emergence cages. Calipers for wing measurements.

Ground:

Experiment Identification Code: DB-n(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Jacqui van Twest

The Bionetics Corporation
Mail Code BIO-3
Kennedy Space Center, FL 33899

Critical Question(s) Addressed:

DB-1, DB-2, DB-3

3. Experiment Title

Effect of Microgravity on Chick Pre-Cardiac Differentiation and Heart Development.

4. Purpose/Hypothesis

Microgravity will adversely effect pre-cardiac differentiation by interfering with myofibrillogenesis.

5. Scientific Rationale/Rationale for Microgravity

It is known that muscle degenerates during extended periods of spaceflight. Similar effects are seen in patients experiencing extensive bedrest. At the macromolecular level of muscle biochemistry the dynamics effected are unknown. Therefore, by studying a process which on Earth requires primarily one side of the equation - the synthesis of muscle, may help target 1g dependent areas of muscle dynamics.

6. Approach

A. Experimental Duration: (A) 4 days for organ culture of pre-cardiac tissue and (B) 30 days storage in the microgravity environment.

B. General Approach

Description:

Part A: would involve organ culture of pre-cardiac tissues, pre-flight, storage at cooler temperatures to impede development, followed by activation in flight (by elevating the temperature) and fixed 4 days after activation.

Part B: Fertile chick eggs would experience a fixed time in microgravity at the reduced temperature and brought back to Earth. Development would be initiated post-flight and terminated at 1, 2, 4, 10 and 21 days of incubation.

C. Number and Type of Specimen:

Part A: At least 40 organ cultures to allow study of the three muscle specific proteins as well as electron microscopy. Part B: 30 eggs, to allow for five embryos at six time points during development.

D. Measurements/Sample

Preflight: Part A: Dissection of pre-cardiac tissue and set up of organ culture, keep cool until in the spaceflight environment.

Part B: Cool fertilized chick eggs to arrest development.

In-flight:

Part A: Activate experiment by increasing the temperature to 38°C

Part B: Keep eggs at the cooler temperature, throughout the 30 days.

Postflight: Part A: Ten samples will be used for electron and light microscopy will be used to assess morphology, myofibrillogenesis and muscle specific protein distributions.

Part B: Following incubation embryos will be sacrificed for analysis. Morphology will be documented and the heart tissue analyzed by electron and light microscopy.

E. Specific Sample Analysis:

In-flight: Record incubator temperature and humidity for complete records of the environmental conditions. This data may also be used on delayed ground controls to mimic conditions of flight samples.

Postflight:

Light and electron microscopy will be the primary tools used to assess morphology, myofibrillogenesis and muscle specific protein distribution(s).

F. Experimental Controls:

In-flight: 1g on-board centrifuge

Ground: (1) Eggs and organ cultures will experience forces similar to those associated with launch and landing. (2): Eggs and organ culture will not experience simulated launch and landing. Both sets of controls will be housed in hardware identical to the flight hardware. Ground control experiments follow identical pre- and post- flight conditions.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: (a) Incubators with the following capabilities; temperature control from 4-40°C (if housing is to be kept the same), rotation, and humidity control.
(b) 1g centrifuge also having capabilities equal to the incubator.

Ground: Microscopes (dissecting and inverted) with cameras, dissection lamps and general laboratory equipment (e.g. beakers), chemicals etc. Lab containing sink, fume hood, bench space. Depending on location of the ground control, this list could be extensive if processing occurred away from the PI laboratory.

Experiment Identification Code: DB-o(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Arleen B. Rifkind, M.D.

Cornell University Medical College
1300 York ave.
New York, NY 10021

Critical Question(s) Addressed:

DB-1

3. Experiment Title

Effect of Weightlessness on Hepatic Cytochrome P450 Function.

4. Purpose/Hypothesis

This experiment will test the hypothesis that weightlessness will affect the expression of hepatic cytochrome P450 dependent drug metabolizing enzyme activity.

5. Scientific Rationale/Rationale for Microgravity

The hepatic cytochrome P450 system provides the body's main mechanism for metabolizing drugs and other foreign chemicals. It also is involved in metabolizing endogenous compounds including steroid hormones and lipids. The cytochrome P450 system is subject to regulation (induction and inhibition) by environmental influences including stress. It will be of interest to learn whether changes in gravity affect P450 function. The question is important to address because changes by weightlessness in P450 function would have significant clinical implications for drug treatment of astronauts traveling in space.

6. Approach

A. Experimental Duration: 18 days.

B. General Approach

Description:

Fertile chicken eggs will be incubated in flight. After landing, livers will be removed and the microsomal fractions prepared and assayed for cytochrome P450 function and distribution using established enzymic assays and Western blotting techniques. Controls will be embryos from the same strain of chickens incubated on earth

C. Number and Type of Specimen:

40 eggs incubated in flight, and 40 controls

D. Measurements/Sample

Preflight: None, fertilized chicken eggs will be cooled to arrest development.

In-flight: Initiate development by placing eggs in incubator 18 days before landing.

Postflight: Livers will be removed and processed to make microsomal fractions and then frozen at -80°C.

E. Specific Sample Analysis:

In-flight: None

Postflight: Preparation of liver microsomes, enzymic assays of cytochrome P450 function; SDS-polyacrylamide gel electrophoresis and Western blotting for P450 content.

F. Experimental Controls:

In-flight: None

Ground: Controls from the same strain of chickens, incubated for the same period.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Egg incubator

Ground: Egg incubator (for controls), dissecting equipment, homogenizers, mid speed and high speed (ultra) centrifuges and a -80C freezer to prepare and store liver microsomes. Microsomes when prepared would be sent for analysis to a laboratory with expertise in studying avian P450 function.

Experiment Identification Code: DB-p(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Cesar D. Fermin

Department of Pathology and Lab Medicine
1430 Tulane Ave./ SL 79
Tulane Medical School
New Orleans, LA 70112-2699

Critical Question(s) Addressed:

DB-2, DB-3

3. Experiment Title

Effect of Weightlessness on Peripheral and Central Afferent Vestibular Innervation.

4. Purpose/Hypothesis

Afferent neurons that carry information from gravity receptors of the inner ear to brain are induced to sprout when exposed to microgravity.

5. Scientific Rationale/Rationale for Microgravity

Detection by vertebrates of the ever present 1.0G force vector on Earth, is greatly dependent on the gravity detecting organs, saccule and utricle. Previous experiments in rodents and birds suggest that afferent neurons may be induced to increase their number of dendrites inside these organs. An increase in the number of branches is an indirect indication of: a) an increase number of synaptic contacts of dendrites with receptors, or b) a modification of the ratios of active to latent synapses. Both ideas deserve testing (morphology and physiologically, See Dr. Jones Ref. Exp.) and can only be done in space (microgravity) with controlled 1.0G rotation experiments side by side, because on earth, gravity can be increased but not significantly reduced.

6. Approach

A. Experimental Duration: 09-30 days.

B. General Approach

Description:

A multidisciplinary approach will be used, which can include physiological measurements of vestibular evoked responses, morphometric measurements (histology and EM) and molecular assays. Fertile eggs will be exposed to microgravity, and the 1.0 G force of an on-board centrifuge. One half of the embryos will be fixed in flight and the remaining embryos will be returned to Earth and fixed upon landing. The returning embryos could be synchronized to hatch near landing and physiological measurements coordinated with Dr. Jones' technique for measuring vestibular evoked responses. With/without physiological measurements, the embryos would be sacrificed and inner ear/brain tissues examined for dendritic branching with a battery of histological, immunohistological and molecular approaches. Except for the eyes and the lower mandible. the temporal bones and brain structures connecting to the VIIIth nerve are required.

C. Number and Type of Specimen:

The incubator for the proposed experiments should accommodate 30-50 fertile chicken or quail eggs. This number allows for casualties and provides a minimum $n=7$ (required for proper statistical analyses) of at least three groups. One half of the specimens will be fixed in flight using the same method of double bag containment that was recently used in the SLM-1 experiments. The remaining eggs will be returned to Earth for measurements or specimen processing.

D. Measurements/Sample

Preflight: 1-Fertilized eggs could be cooled to arrest development.
2-Fertilized eggs could be pre-incubated to start development before flight.
3-Fertilized eggs could be divided, half for each above.

In-flight: Switching on/off of the incubator may be needed. Temperature and humidity control should be automatic. In addition, vibration and radiation should be controlled as much as possible including very low frequency magnetic fields.

Postflight: Functional testing may be required depending on final experimental design and inter-investigators interactions. Collection of tissues required.

E. Specific Sample Analysis:

In-flight: Automatic incubation timing. No crew participation required during incubation.

Postflight: The temporal bones and the brain stem nuclei that receive afferent inputs will be examined for dendritic branching with a battery of histological, immunohistological and molecular approaches. Measurements will include: T-test of numerical counts of dendritic branches between flight (uG) vs. flight (1.0G) and the above to synchronous 1.0G earth controls. Physiological measurement may be required if combined with another experiment.

F. Experimental Controls:

In-flight: For testing the hypothesis and arriving at any meaningful comparisons, a 1.0G centrifuge in flight would be desirable to eliminate variables other than microgravity, and which could affect development.

Ground: A pre-flight 1.0G vibration and acceleration control is needed plus the synchronous control to the above in-flight groups.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Incubator with capability to control temperature at 38C and humidity.

Ground:

Experiment Identification Code: DB-q(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Gary W. Conrad

Division of Biology-Ackert Hall
Kansas State University
Manhattan, KS 66506-4901

Critical Question(s) Addressed:

DB-2, DG-2

3. Experiment Title

Effects of Microgravity on Intraocular Pressure (IOP) During Embryonic Eye Development.

4. Purpose/Hypothesis

1. Embryonic development of the eyes of chickens or quail will appear to be normal when assessed by gross measurements of weight and diameter. 2. However, Intraocular Pressure (IOP) of in-flight embryos will be > 50% higher than controls immediately after exposure to microgravity, but then will decrease gradually toward control levels. 3. In addition, IOP of in-flight embryos will be >50% lower than controls immediately upon return to Earth, but then will increase gradually toward control levels.

5. Scientific Rationale/Rationale for Microgravity

IOP of adult humans increases by more than 50% within 20 sec after exposure to microgravity. Normal embryonic development of the vertebrate eye requires the constant presence of IOP. If IOP increases when embryos are exposed to microgravity, it is likely that such high pressure may tend to decrease toward control levels after a few days of adjustment to microgravity; conversely, when such embryos are returned to Earth, it seems likely that their IOP's will be abnormally low for a few days, but then will tend to rise to control levels gradually.

6. Approach

A. Experimental Duration: Chicken eggs:20 days/quail eggs:16 days, both maxima.

B. General Approach

Description:

1. Fertilized chicken or quail eggs will be incubated on earth for 1-4 days, then divided into 3 groups: Flight, Ground Control (GC)(Flight style incubator on Earth), and Control (Con)(standard poultry incubator on Earth). 2. Flight embryos will be exposed to microgravity for 7-14 days; determine IOP's immediately upon reaching microgravity and at 1 or 2-day intervals while in microgravity and return remaining embryos to Earth; simultaneously, determine IOPs similarly in each of the two control groups. 3. Upon return of flight embryos to Earth, determine IOPs immediately in some remaining flight, GC and Con embryos, and at daily intervals thereafter until hatching. 4. Determine IOPs by mechanical transduction of the cornea in situ (via shell window).

C. Number and Type of Specimen:

30 fertilized eggs (White Leghorn Chicken or Japanese Quail) needed for each three groups (Flt, GC, Con) = 90 fertilized eggs total

D. Measurements/Sample

Preflight: None

In-flight: IOP measurements.

Postflight: IOP measurements.

E. Specific Sample Analysis:

In-flight: None

Postflight: None

F. Experimental Controls:

In-flight: None, but embryos in a 1-g centrifuge would be an excellent control.

Ground: Eggs in a flight-style incubator + a standard poultry incubator.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Flight-style + standard poultry incubators (rocking)

Ground: Flight-style + standard poultry incubators (rocking)

Experiment Identification Code: DB-r(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Russell L. Kerschmann

Assoc. Prof. Pathology
University of California at San Francisco
Dept. Pathology, Building 3
San Francisco General Hospital
San Francisco, CA 94110

Critical Question(s) Addressed:

DB-1, DB-2, DB-3, DG-1

3. Experiment Title

Effects of Microgravity on the Morphology of the Vestibular End Organ.

4. Purpose/Hypothesis

It is hypothesized that the development of the topology of the vestibular labyrinth is influenced by gravitational force as well as by genetic factors. The experiment studies the effect of gravity on the final shape of this important element of the vestibular end organ.

5. Scientific Rationale/Rationale for Microgravity

Sensory stimulation is an important factor in the normal ontogeny of the visual and auditory systems, but little is known about the effects of the environment on vestibular development. In particular, is the precise shape of the membranous labyrinth in any way influenced by gravity during critical periods in the formation of this organ? Since even subtle alterations in the topology of the semicircular canals, utricle and other endolymph-containing components could have profound effects on functional fluid dynamics, precise morphometric data should be obtained from animals developed in space, and compared to those raised under 1.0-g and hypergravity control conditions.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

The onset of incubation should be delayed until the eggs are in orbit so that the entire developmental period of the embryos will occur in microgravity. In order to preserve the precise anatomic configuration of the delicate membranous labyrinth, endolymphatic perfusion fixation of newly hatched chicks under physiologic pressures should be performed, ideally on board ISS. If this is not possible, then the eggs should be returned to Earth just prior to hatch for immediate processing.

C. Number and Type of Specimen:

20 microgravity and 20 in-flight 1-g centrifuge control animals.

D. Measurements/Sample

Preflight: Fertilized chicken eggs will be cooled to delay onset of development.

In-flight: If hatching and perfusion can be performed on board ISS, sufficient additional time (2 days) should be allowed for processing the material.

Postflight: A temporary laboratory at the landing site will be required for perfusion fixation.

E. Specific Sample Analysis:

In-flight: No intervention required during incubation. In-flight post-hatch labyrinth perfusion fixation desirable, but not essential.

Postflight: Tissues will be processed to very high resolution three dimensional computer models. Quantitative comparison of microgravity and 1-g material will reveal to the 1 micron level, any repeatable differences due to lack of gravity during development. Models can be computationally averaged to minimize individual variation among the experimental groups. Subsequent fluid dynamic simulation studies will be performed on the models to gain insight into the functional consequences of any anomalies.

F. Experimental Controls:

In-flight: 1-g centrifuge controls.

Ground: 1) Synchronous ground controls with simulated launch and landing gravitation profiles.
2) Normal ground controls. 3) High-g centrifuge controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

Experiment Identification Code: DB-s(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Jose M. Icardo

Department of Anatomy and Cell Biology
University of Cantabria
39011-Santander
Spain

Critical Question(s) Addressed:

DB-2, DB-3, DA-6

3. Experiment Title

Effects of Weightlessness on Heart Loop Formation and Late Cardiac Development.

4. Purpose/Hypothesis

This experiment will test the hypothesis that depriving embryos of a steady gravitational field will modify the direction of the cardiac loop and result in development of heart malformations.

5. Scientific Rationale/Rationale for Microgravity

Formation of the heart loop is the first morphological expression of the establishment of right/left asymmetry. Proper formation of the heart loop is essential to late heart development. Applying pressure (or releasing tension) on the embryonic body produces inversion of the heart loop. Abnormal and/or inverted loops result in a large number of heart malformations. It is expected that under 0-g conditions right/left heart asymmetry will be abnormal and hearts will develop a number of malformations.

6. Approach

A. Experimental Duration: 0 - 10 days.

B. General Approach

Description:

Fertile chicken eggs will be incubated in-flight. Animals will return to Earth on days 2, 5 and 10 of development. The hearts will be examined in situ and processed to evaluate morphological changes.

C. Number and Type of Specimen:

36 fertilized eggs.

Additional samples if 1-g centrifuge is available.

D. Measurements/Sample

Preflight: Fertilized chicken eggs will be cooled to arrest development.

In-flight: Incubation temperatures will be raised at the appropriate time(s) to initiate development.

Postflight: Study of the morphological characteristics of the embryonic hearts.

E. Specific Sample Analysis:

In-flight: Automatic incubation time to give 2, 5 and 10 days embryos. If different incubation times are not available, automatic incubation time to give 10-day embryos.

Postflight: Study of the morphological characteristics of the embryonic hearts.

F. Experimental Controls:

In-flight: 1-g centrifuge controls if centrifuge is available. Otherwise, none.

Ground: Controls will parallel flight experiments, including simulated lift-off and landing conditions.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Incubator

Ground: Routine morphological laboratory equipment.

Experiment Identification Code: DB-t(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Jacqui van Twest

The Bionetics Corporation
Mail Code BIO-3
Kennedy Space Center, FL 33899

Critical Question(s) Addressed:

DB-1, DB-2, DB-3, DA-2, DG-1

3. Experiment Title

Effects of Weightlessness on the Early Stages of Chick Heart Development.

4. Purpose/Hypothesis

These experiments will test the hypothesis that a microgravity environment affects early cardiac muscle formation and development.

5. Scientific Rationale/Rationale for Microgravity

All life as we know it has developed under the influence of the gravitation field exerted by the Earth's rotation. The effects of the gravitational vector on developing biological systems are not fully understood. Spaceflight has been found to adversely effect both quail and chick development. Most notably embryos developing from day zero in a reduced gravity environment. Extended periods of microgravity are also know to cause muscle atrophy and decreased cardiac workload. Therefore, it seems feasible that development of the heart in an altered gravity environment may result in a different developmental profile compared to development and differentiation seen on Earth.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

Avian embryos will be exposed to zero and one gravity environments. Development will be initiated by incubation at the applicable temperature (for chick eggs this is 38°C). At 1, 2, 4, 10 and 21 days of incubation development will be terminated using fixative, providing a profile of chick development in microgravity. To maximize efficiency, tissues would be distributed to scientists to examine.

C. Number and Type of Specimen:

At least 4 embryos from each of the time points (i.e. a minimum of 24 eggs). This would allow valid statistical evaluation.

D. Measurements/Sample

Preflight: Fertilized chick eggs will be stored at a reduced temperature (12°C) to arrest development.

In-flight: Development could be initiated by either (1) transfer into an incubator at a higher temperature or (2) simply increasing the temperature in the launch container. Maintenance of humidity and rotation of eggs is vital.

Postflight: Analyze the heart tissue using light and electron microscopy to assess overall morphology, myofibrillogenesis and muscle specific protein patterns (e.g. actin and tubulin).

E. Specific Sample Analysis:

In-flight: Record incubator temperature and humidity for complete records of the environmental conditions. This data would allow time-delayed ground controls experience conditions closer to those of the flight samples.

Postflight: Light and electron microscopy will be the primary tools used to assess myofibrillogenesis and muscle specific protein distribution(s).

F. Experimental Controls:

In-flight: 1g on board centrifuge

Ground: Control Set A: Eggs will experience forces similar to those associated with launch and landing. Control Set B: Eggs will not experience simulated launch and landing. Both sets of controls will be housed in hardware identical to the flight hardware. Eggs will experience similar pre- and post-flight conditions.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: (a) Incubators with the following capabilities; temperature control from 4-40°C (if housing is to be kept the same), rotation, and humidity control.
(b) 1g centrifuge also having capabilities equal to the incubator (a).

Ground: Microscopes (dissecting and inverted) with cameras, dissection lamps and general laboratory equipment (e.g. beakers, chemicals etc). Depending on location of the ground control studies and post flight analysis this list could be extensive. If processing occurred away from the PI laboratory everything from laboratory containing

Experiment Identification Code: DB-u(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. G. Causey Whittow

Department of Physiology
John A. Burns School of Medicine
University of Hawaii
1960 East West Road
Honolulu, HI 96822

Critical Question(s) Addressed:

DB-2, DB-3, DG-5

3. Experiment Title

Embryonic Organ Growth in a Weightless Environment.

4. Purpose/Hypothesis

Embryonic organ growth is susceptible to a number of environmental conditions including temperature and electromagnetic fields. It is believed that the absence of a steady gravitational field will have differential effects on the growth of avian embryonic organs and tissues.

5. Scientific Rationale/Rationale for Microgravity

During embryonic development, the postural muscles have to develop in order to permit the hatched chick to stand and walk, and also play an important part in thermoregulation. Is the pattern of organ and tissue development different in a weightless environment?

6. Approach

A. Experimental Duration: 7-22 days.

B. General Approach

Description:

Groups of avian embryos will be incubated during the flight, in gravitational fields varying from 0- to 1- g. Samples of eggs will be removed from each group, at regular intervals, wrapped in water-retaining plastic, and stored at low temperature. On return to earth, the embryos will be carefully dissected. The weights of the constituent organs will be measured together with their water content determined as indicator of tissue age.

C. Number and Type of Specimen:

Incubators should be able to accommodate 60 fertilized eggs.

D. Measurements/Sample

Preflight: Fertilized chicken eggs will be cooled to arrest development.

In-flight: Incubation will commence; samples of eggs (10) will be removed at regular intervals (every other day after day 10).

Postflight: Organ and whole embryo weights and water contents will be measured.

E. Specific Sample Analysis:

In-flight: Automatic incubation, egg turning. Manual removal of egg samples and storage at low temperature.

Postflight: Dissection of embryos; measurement of organ weights and water content.

F. Experimental Controls:

In-flight: 1-g centrifuge controls.

Ground: The experiments performed in flight will be repeated using the same gravitational fields used in flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Incubator, simulated gravitational fields.

Ground: Incubator, refrigerator, simulated gravitational fields, balance.

Experiment Identification Code: DB-v(2) D = developmental; B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Stephen B. Doty

R 424 Research Building
The Hospital for Special Surgery
535 E. 70th Street
New York, NY 10021

Critical Question(s) Addressed:

DB-2, DB-3, DG-2

3. Experiment Title

Musculoskeletal Development in a Microgravity Environment.

4. Purpose/Hypothesis

Reduction of mechanical stimulus during embryogenesis, as would occur in the microgravity of space, will result in abnormal limb development and incomplete skeletogenesis.

5. Scientific Rationale/Rationale for Microgravity

There is a genetic and mechanical component to normal development of the musculoskeletal systems. Genetically driven skeletal tissue or limb development may produce abnormal tissues in microgravity which may or may not be reversible as embryogenesis advances in the whole embryo.

6. Approach

A. Experimental Duration: Quail eggs -- incubate to hatching (17 days).
Chick eggs --incubate to hatching(18/21 days).

B. General Approach

Description:

Eggs can be refrigerated prior to launch and then replaced into an incubator when orbit is reached. Incubation times can be varied to provide greatest information per each investigator and specific organ/tissue of interest. Egg can be opened within a containment bag which contains preservative. These bags can be stored until return to Earth.

C. Number and Type of Specimen:

Each time period requires a minimum of 5 eggs for necessary statistical evaluation. A single experiment could require 30 eggs or more.

D. Measurements/Sample

Preflight: Eggs refrigerated prior to and during launch.

In-flight: Eggs placed into containment bags already filled with preservative. Eggs cracked after placing into preservative but with bags closed to contain fixative.

Postflight: All sample handling done in laboratory following flight; either at NASA site or within investigators own lab.

E. Specific Sample Analysis:

In-flight: None

Postflight: Physical measurements of body, limbs, etc. Observations of gross abnormal development; some photographic records. X-ray of whole body for skeletal development and size. Histology, immunocytochemistry, histochemistry, cell and molecular biology measurements.

F. Experimental Controls:

In-flight: A 1 X g centrifuge might provide useful controls if it can be designed to work with different size eggs.

Ground: Age matched embryos necessary. Embryos will have to be exposed to vibration and hyper-g encountered during launch conditions. Any temperature variations encountered during flight will have to be reproduced in controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Incubator. Refrigeration also necessary; either as part of incubator or separate.

Ground: Same incubator used for flight will have to be available for ground controls. Refrigeration also necessary; either as part of incubator or separate.

Experiment Identification Code: DB-w(2) D = developmental, B = later developmental events

1. Discipline: Developmental Biology

2. Author(s): Dr. Fredric J. Janzen II

Department of Zoology and Genetics
Iowa State University
Ames, IA 50011

Critical Question(s) Addressed:

DB-2, DB-3, DA-3

3. Experiment Title

The Role of Microgravity in Sex Determination of Embryonic Reptiles.

4. Purpose/Hypothesis

The absence of gravity could disrupt the cell fates and differentiation of many organ systems. This experiment will test the hypothesis that gonads will develop abnormally from kidney tissue.

5. Scientific Rationale/Rationale for Microgravity

Proper organogenesis and development of various anatomical structure is critical for producing viable animals. Turtles with temperature-dependent sex determination offer an excellent model system to evaluate this process in the absence of gravity. Because gonadal sex can be manipulated simply by altering incubation temperature, one can evaluate expected sex ratios and sexual development under 0-g conditions without confounding genetic effects. Such a study would provide excellent information on organogenesis in a critical system for reproduction.

6. Approach

A. Experimental Duration: 60-90 days, depending on incubation temperature.

B. General Approach

Description:

Incubation of 20 fertile eggs of red-eared slider turtles at a male-producing temperature (~25°C) and 20 additional eggs at a female-producing temperature (~30°C). Sex ratios and gonadal morphology will be evaluated post-flight.

C. Number and Type of Specimen:

40 fertilized turtle eggs.

D. Measurements/Sample

Preflight: Fertilized turtle eggs will be obtained from a commercial turtle farm and will be placed on the shuttle the day of the flight.

In-flight: Eggs will be maintained in constant temperature conditions. Water will be added once weekly to keep the incubation substrate moist.

Postflight: Gender and gonadal morphology of hatchling turtles will be assessed.

E. Specific Sample Analysis:

In-flight: None other than automatic temperature control.

Postflight: Sex will be determined by dissection. Gonadal morphology will be compared to that of Earth-incubated turtles.

F. Experimental Controls:

In-flight: None

Ground: Normal laboratory controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: 2 incubators

Ground: Fixative, dissecting microscope, histological fees

Experiment Identification Code: DC-a(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Richard S. Nemeth

Department of Zoology
University of New Hampshire
Durham, NH 03824

(206) 862-2954

Critical Question(s) Addressed:

DC-4, DG-4, DB-2, DE-2

3. Experiment Title

Using Microgravity to Examine the Structure and Function of Otolith Bones in the Inner Ear of Teleost Fishes.

4. Purpose/Hypothesis

The purpose of this study is to determine if the development of otoliths of teleost fishes is sensitive to the effects of earth's gravity. Because otoliths function in orientation and hearing of fishes, it is hypothesized that gravity may play an important role in determining otolith morphology.

5. Scientific Rationale/Rationale for Microgravity

All teleost fishes have three pairs of otoliths (sagittus, lapillus, astericus) which rest on a bed of sensory hairs in the labyrinth of the inner ear. When the otoliths are disturbed, due to sound waves traveling through water or to the repositioning of a fish's body, the sensory hairs are stimulated and transmit information to the brain. Because otoliths are used in orientation, their structure may be sensitive to the effects of gravity. Since otoliths form at hatching and continue to grow through adulthood by the daily deposition of a calcium matrix, fish raised in microgravity may orient to their surroundings differently than fish raised at normal 1-g conditions and subsequently may develop differences in their otolith microstructure. At present, it is unknown how otolith morphology contributes to orientation and sound detection. These studies will provide valuable information on the relationship between form and function of an important sensory system in teleost fishes.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Fishes that readily spawn in captivity (i.e. cichlids, zebrafish) are ideal for this study. After eggs are laid the brood will be divided with a third of each brood being raised in 0-g, 1-g, and 2-g force environments. This protocol will minimize genetic variability. At the end of 90 days half of each experimental group will be used to examine otolith morphology. The remaining siblings will be placed in 1-g (control) aquaria and compared for differences in behavior, orientation and sound detection.

C. Number and Type of Specimen:

At least 6 pairs of reproductively active cichlids or zebrafish. One advantage of using cichlids is that they lay a single monolayer of eggs which can be easily manipulated.

D. Measurements/Sample

Preflight: One set of offspring from each of 6 pairs of fish will be raised under identical conditions. After 90 days, half of each clutch will be used to examine otolith microstructure and half will be used to examine differences in behavior, orientation, and sound detection.

In-flight: In-flight: 3 adult pairs will be randomly selected for in-flight tests. The first clutch of each pair will be divided, a third of each egg clutch being raised in 0-g, 1-g, & 2-g force environments. Orientation of juveniles will be monitored periodically during the experiment. After 90 days all offspring will be returned to earth.

Postflight: Postflight: Half of each experimental group will be used to examine otolith microstructure. The other siblings will be placed in 1-g (control) aquaria and observed for differences in behavior, orientation and sound detection. After 30 days these fish will be used to compare otolith microstructure to ground controls of similar age.

E. Specific Sample Analysis:

In-flight: None

Postflight: All otoliths will be dissected from fish, rinsed in distilled water, dried and weighed. Otolith microstructure (i.e. area, perimeter, radius, center of mass, shape, width of daily otolith increments) will be measured and quantified with video image analysis system.

F. Experimental Controls:

In-flight: Use 1-g force on one third of each brood.

Ground: Same as in-flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Aquaria with controlled temperature and lighting.

Ground:

Experiment Identification Code: DC-b(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): James S. Diana

School of Natural Resources and Environment,
University of Michigan
Ann Arbor, MI 48109-1115

(313) 763-5834

Critical Question(s) Addressed:

DE-2, DG-4, DB-3, DC-4, DG-1

3. Experiment Title

Influence of Reduced Gravity on Buoyancy, Swimming, and Muscle Development in Fish.

4. Purpose/Hypothesis

Under reduced gravity development will occur with altered water viscosity, pressure, and fish buoyancy. All of these factors influence swimming performance in young fish, which in turn should influence body composition (muscle mass and lipid stores). The null hypothesis is that swimming performance and early growth will not be altered under low gravity.

5. Scientific Rationale/Rationale for Microgravity

Fishes swim by overcoming the viscosity of water and friction of their movements. Swimming form is dependent on Reynold's number, which is a function of fish length, swimming velocity, and water viscosity. Adult fish have high Reynold's numbers, and use swimming methods to overcome inertial forces. Larval fish have low Reynold's numbers and must use methods to overcome viscosity. Body musculature, flexibility and swimming performance should vary with alterations in gravity, influencing body form, growth and energy storage.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Fish will be cultured from fertilized eggs. Video taping of normal movements will be done regularly. Samples of fish will be collected at yolk sac absorption, 45 d, and 90 d for tissue analysis. Caloric content, protein and lipid content will be analyzed.

C. Number and Type of Specimen:

A small fish (medaka, zebrafish) will be used. Up to 50 young will be utilized. Samples of 10 fish will be removed at each sampling interval.

D. Measurements/Sample

Preflight: Mate adults and produce fertilized eggs.

In-flight: Expose fertilized eggs in water. Video tape swimming behavior continuously. Remove individuals for later analysis.

Postflight: Evaluate video tape for swimming mechanics, especially for flexure, speed and duration. Evaluate body composition for the differences between reduced gravity and controls.

E. Specific Sample Analysis:

In-flight: Freeze samples when collected. Store video tapes.

Postflight: Tissue analysis by calorimetry, lipid extraction and protein analysis. Also evaluate body shape and form.

F. Experimental Controls:

In-flight: 1-g control.

Ground: 1-g control.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Aquaria at reduced and 1-g in flight. Freezer storage. Video taping for each aquarium.

Ground: Similar requirements for ground control.

Experiment Identification Code: DC-c(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Michael L. Wiederhold,

Dept. Otolaryngology - Head & Neck Surgery
University of Texas Health Science Center
7703 Floyd Curl Dr.
San Antonio, TX 78284-7777
(210) 567-5655 Fax: (210) 567-3617

Critical Question(s) Addressed:

DC-4, DB-2, DG-4, DE-2, DG-2

3. Experiment Title

Development of Gravity-sensing Organs in Long-term Microgravity.

4. Purpose/Hypothesis

It is not known what mechanisms control the growth of the otoliths, test masses upon which gravity and linear acceleration act. If the control is based on the otolith weight, one would expect larger otoliths to be formed in u-g. Otolith-ocular reflexes, which stabilize images on the retina, normally develop with a constant gravitational pull on the otoliths. Without this constant input, it is of interest to determine if these reflexes will develop normally.

5. Scientific Rationale/Rationale for Microgravity

Preliminary results of our experiment on IML-2 indicate that, within one week after a 15-day development period in u-g, the saccular and utricular otolith volumes are not significantly different between space- and ground-reared specimens. However, the endolymphatic sac and volume of otoconia within the sac are significantly larger in space-reared specimens. Amphibians store metabolic calcium in the endolymphatic otoconia. The excess endolymphatic otoconia produced a much larger saccular otolith in flight-reared larvae several months post flight, as revealed by X-ray microfocus studies performed in Japan. The increase in endolymphatic otoconia appeared at 3-5 days after recovery, raising the question of whether the increase would occur with extended time in u-g or is a reaction to the first exposure to 1-g conditions.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Fertilized eggs of the Japanese newt, *Cynops pyrrhogaster*, will be flown in an Aquatic Habitat. Eggs will be selected such that they will reach u-g (in orbit) before any of the otoconia in the inner ear are formed. Eggs will be allowed to develop for 20 to 90 days in orbit to compare long vs. short terms in u-g. Post-flight, volume of otoliths and area of sensory maculae/cristae will be measured from 3-d reconstructions of sectioned, fixed specimens. The otolith-ocular reflex will be assessed in live specimens upon recovery and at weekly intervals post-flight. In collaboration with Japanese investigators, images of otoliths, including the endolymphatic system, will be obtained at multiple times in specimens maintained up to 5 months post-flight.

C. Number and Type of Specimen:

200 fertilized eggs of the Japanese red-bellied newt, Cynops pyrrhogaster, at development stages 15 to 25 (4 to 6 days after eggs are laid) at launch.

D. Measurements/Sample

Preflight: Adult female newts, collected in hibernation after the fall mating, will be transported to the launch site and maintained in hibernation at 4°C. Fertilization and spawning is accomplished by warming the females to 22°C and injecting them with hormone.

In-flight: Eggs are laid 2-4 days after hormone injection.

In-flight: Eggs will be observed & video taped, with down-link, to observe their rate of development. A group of 50 eggs will be removed from the aquatic system at 20 days post launch and another 50 removed after 50 days on station. These eggs will be

Postflight: fixed in flight for post-flight anatomical analysis.

Postflight: The gain of the otolith-ocular reflex will be measured in 20 larvae, both within 1 day postflight & 1 week later. 20 will be fixed immediately upon retrieval & 20 will have their otoliths & endolymphatic system assessed with the Japanese X-ray microfocus system at biweekly intervals for 6 months postflight. 10 each will be fixed for anatomical study at one and 3 months postflight.

E. Specific Sample Analysis:

In-flight: Observe developmental stage via high-magnification video recording with down-link.

Postflight: Test a sample of larvae for gain of otolith-ocular reflex. Sequentially measure area and volume of saccular, utricular and endolymphatic otoliths/otoconia with X-ray microfocus system. As samples are sacrificed, fix, embed and cut serial sections for 3-D reconstruction. Perform FTIR spectroscopy on sample otoconia to determine crystal form of CaCO₃. Observe locomotion patterns and body/head posture.

F. Experimental Controls:

In-flight: None

Ground: An aquatic Habitat functionally identical to that on the Space Station will be maintained in the P.I's laboratory to simulate the environmental conditions of the experimental group. A third set of larvae will be reared in laboratory dishes at the same temperature as the light and ground-control specimens.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: The facility must be able to support 200 developing eggs and larvae with freely flowing aerated water and, after the first 2 weeks, when the animals are nourished by their yolk sac, the larvae will have to be fed.

Ground: Ground-controls will be run at the PI's lab, with internet or some equivalent electronic communication between the station, Control center and the P.I's Lab.

Experiment Identification Code: DC-d(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Daniel Weber

Marine & Freshwater Biomedical Sciences Center
University of Wisconsin, 600 E. Greenfield Ave.,
Milwaukee, WI 53204

(414) 382-1726

Critical Question(s) Addressed:

DE-2, DG-4, DG-1, DC-4, DB-1, DB-2

3. Experiment Title

Effects of Microgravity on Cardiovascular Function in Fish.

4. Purpose/Hypothesis

The purpose of this experiment is to determine the effects of microgravity on sustained, forced swimming abilities against a unidirectional flow. The potential effects may include maximum swimming velocity, orientation abilities within current, heart morphology, hematocrit levels and hemoglobin production, gill histology and respiratory capacity.

5. Scientific Rationale/Rationale for Microgravity

Fish body contours are adapted for swimming in a dense medium. Sustained movement through that medium is dependent on water velocity, cardiovascular capacity which includes heart function, blood variables and gill structure, and positive rheotropic responses, i.e. orientation upstream. Using the well-used concept of critical swimming velocity, maximum velocity for sustained swimming can be determined. Since microgravity may be a potential stressor and may alter heart function, blood composition or gill structure, it is reasonable to assume that sustained swimming capacity could be affected. Additionally, since microgravity may alter neuron function and/or alter orientation abilities, rheotropic responses, swimming abilities against a current may be changed simply because the fish is unable to maintain a proper position within the water column.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

Following standard designs for swimming tunnels for fish, fish will be forced to swim against a unidirectional flow of water at consecutively higher velocities until, due to exhaustion, it can no longer maintain its position and it ceases to swim. Fish of different age-classes will be used with each fish acting as its own control, i.e. it is retested to determine long-term effects.

C. Number and Type of Specimen:

15, adult, 15 juvenile fathead minnows.

D. Measurements/Sample

Preflight: Length, weight recorded for each fish. Place fish into swim chambers to acclimate them to surroundings. Maintain very low water velocity to acclimate to water movement and to maintain water quality.

In-flight: Critical swimming velocity determined for each fish. Test on days 1, 8, 15, 30. Observations on orientation abilities (rheotropic, body position relative to "normal" dorso-ventral presentation) recorded. 5 adults and 5 juveniles to be preserved in formalin on day 30 for histological analyses.

Postflight: Repeat test on remaining fish after one week acclimation to determine if behavior and physiology return to normal. Preserve 5 adults and 5 juveniles in formalin. Take blood sample for hematocrit analysis from remaining fish. Length recorded for all fish.

E. Specific Sample Analysis:

In-flight: None

Postflight: Calculate critical swimming velocity for all fish normalizing to body lengths/sec. Prepare histological slides for heart and gill analyses. Determine hematocrit levels. Compare data between two age-classes.

F. Experimental Controls:

In-flight: None

Ground: Identical experiment will be conducted.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Special platform module to contain 30 small swimming tunnels (recirculating, filtered, dechlorinated water capable of being adjusted to a wide range of velocities).

Ground:

Experiment Identification Code: DC-e(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Konrad Dabrowski

School of Natural Resources, Ohio State University
2021 Coffey Road
Columbus, OH 43210

614-292-4555

Critical Question(s) Addressed:

DA-3, DA-4, DC-1, DC-3, DG-4

3. Experiment Title

The Effect of Microgravity on Spermatogenesis in Lower Vertebrate.

4. Purpose/Hypothesis

Differentiation of germinal cells during multiple mitotic division of spermatogonia will be pertubated by microgravity. This will effect production of spermatozoa, their viability and fertilizing ability. Frequency of "birth defects" is likely to increase although it remains to be seen how specific these changes will be in comparison to other environmental alterations.

5. Scientific Rationale/Rationale for Microgravity

Germline mutation are likely to occur at a higher rate in sperm than in oocytes. In human sperm development, 380 mitotic divisions take place in comparison to only 23 for the oocyte. In fish, proportion of mititic divisions might be even higher because external fertilization results in prolific production of spermatozoa. If paternal germline mutation is hereditary due to the absence of a DNA repair enzyme in sperm, microgravity perturbation should increase the incidence of genetic defects in progeny.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Young-of-the-year yellow perch (Perca flavescens) males (7-10g individual body weight) will be kept at a long photoperiod (16 h) and above 20° C prior to the experiment. Gradual decreases of water temperature (down to 8° C) and photoperiod (10 h) results in complete spermatogenesis within 90 days. Sperm can be obtained from fish (5-7% body weight) and cold-stored (0° C) or cryopreserved before flow cytometry, motility and fertility tests.

C. Number and Type of Specimen:

50 fish of desired size prior to spermatogenesis.

D. Measurements/Sample

Preflight: Blood samples and testis will be taken from fish before experiment.

In-flight: Blood samples will be collected from caudal vein, centrifuged and frozen for further analysis. Testis will be removed and cryopreserved in buffered - DMSO solution. Eight fish will be sampled at monthly intervals.

Postflight: Flow cytometry, motility (CASA-computer assisted sperm motion analysis) and fertility tests will be performed.

E. Specific Sample Analysis:

In-flight: None

Postflight: Steroids (testosterone, 11-keto testosterone, estradiol) in blood plasma. Germline cells will be stained and analyzed with flow cytometry. Sperm will be collected and tested for fertility or cryopreserved.

F. Experimental Controls:

In-flight: Control fish will be maintained at long photoperiod (16 h) and above 20° C.

Ground: Two groups of fish with (1) induced spermatogenesis (light/temperature manipulated) and controls (16 h light, > 20° C) will be kept.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Temperature/light control units (80 l total volume) with recirculated, UV-sterilization systems.

Ground:

Experiment Identification Code: DC-f(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Konrad Dabrowski and Dr. Feng Lin

School of Natural Resources, Ohio State University
210 Kottman Hall, 2021 Coffey Road
Columbus, OH 43210

Critical Question(s) Addressed:

DA-1, DA-3, DA-4, DC-1, DC-3, DG-4

614-292-4555

3. Experiment Title

Hormonal Profiles and Gamete Quality After Fish being Exposed to the Microgravity Environment.

4. Purpose/Hypothesis

Prolonged exposure to microgravity (a cause of stress) will induce alteration of hormonal profiles and thus affect the gamete quality.

5. Scientific Rationale/Rationale for Microgravity

Precise hormonal regulation is essential for gametogenesis. The hormonal profiles are susceptible to the environmental stresses and are expected to be affected by microgravity conditions. The alteration of hormone levels will affect the process of gametogenesis and quality of its final products, gametes, and thus jeopardize the reproductive success of the fish.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

Fish will be cultured in both 0-g and 1-g conditions and fed with a regular diet. Blood samples will be taken periodically for hormonal analysis by radio-immuno assay (RIA). Gametes will be stripped both in-flight and postflight for gamete quality evaluation. Sperm motility will be evaluated by computer-aided sperm motion analysis (CASA). Sperm chromatin structure and viability will be evaluated by flow cytometry methods. Gamete production will be recorded. Sperm and eggs fertility will be evaluated by the survivals of fertilized eggs to the eyed-stage and hatching.

C. Number and Type of Specimen:

Cloned fish (identical genotype) fish will be used in this experiment to eliminate the variation caused by genotypes. Each group (0-g and 1-g) will contain 12 fish, 6 males & 6 females. Blood samples will be taken weekly, thus a total of 96 samples (4 samples of 24 fish). Gametes will be collected twice (48 samples).

D. Measurements/Sample

Preflight: Prepare cloned goldfish by gynogenesis.

In-flight: Maintain the fish by feeding. Sample blood weekly, prepare & store blood plasma frozen for hormonal analysis. Collect gamete samples for gamete quantity & quality estimation, preserve & store. Fertilize & culture eggs (within groups) for gamete fertility test. Examine the survival of embryos to eyed-stage & hatching.

Postflight: Analyze hormonal levels of blood plasma by RIA. Analyze gamete quality of gamete collected in the Space, given not feasible to carry large equipment, such as flow cytometry, into the space station. Fish will be continuously cultured & monitored for hormonal profiles and gamete viability.

E. Specific Sample Analysis:

In-flight: Prepare & store blood plasma for postflight analysis. Measure the volumes of gametes, egg diameters, & sperm concentrations, & cryopreserve sperm. Perform sperm motion analysis by CASA. Analyze sperm chromatin structure & sperm

Postflight: viability by flow cytometry if equipment is onboard, or cryopreserve sperm samples for postflight analysis.

Postflight: Analyze hormones, steroids and gonadotropins of blood plasma by RIA. Evaluation of gamete quality collected postflight.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Parallel 1-g controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Both 0-g and 1-g fish culture facilities for both adult fish and eggs; supplies for handling fish out of water (for blood samples and gametes collection); microcentrifuge; computer-aided sperm motion analyzer; flow cytometer; spectrophotometer; -196° C storage freezer.

Ground:

Experiment Identification Code: DC-g(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Frieda B. Taub

School of Fisheries
University of Washington
Box 355100
Seattle, WA 98195

Critical Question(s) Addressed:

DE-3, DG-3, DC-2, DC-3, DG-4

3. Experiment Title

Aquatic Habitat.

4. Purpose/Hypothesis

Determine if the self-regulation shown in earth based aquatic habitats function in microgravity. We have shown some capabilities of a 3 phase aquatic habitat to control nitrate, nitrite, and ammonia so as to provide fish survival for > 100 days. We do not know if any of these processes are vulnerable to malfunction under conditions of microgravity.

5. Scientific Rationale/Rationale for Microgravity

Life support systems will be necessary for aquatic animals, if they are to be used in microgravity experiments.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Start with the Aquatic Habitat developed at our laboratory in 1995; develop it and fly it under conditions of microgravity. Flight test the Aquatic Habitat that has shown the capability of supporting fish. Explore the mixtures of organisms that will maximize denitrification to remove N via gas stripping. Optimize the system for fish survival, including smaller fish and protection of eggs from cannibalization.

C. Number and Type of Specimen:

Medaka fish, daphnia, amphipods, ostracods, rotifers, protozqa, algae.

D. Measurements/Sample

Preflight: None

In-flight: Survival.

Postflight: None

E. Specific Sample Analysis:

In-flight: Oxygen, pH, video monitoring of survival, fish swimming, behavior.

Postflight: Nutrients (Nitrate, Nitrite, Ammonia), organism numbers, fish growth, health.

F. Experimental Controls:

In-flight: Ground controls.

Ground:

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Minimum on orbit requirements.

Ground: Hardware requirements for ground.

Experiment Identification Code: DC-h(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Bruce J. Turner

Dept. of Biology
Virginia Polytechnic Inst. & State University
Blacksburg, VA 24061-0406

703-231-7444 Fax: 703-231-9307

Critical Question(s) Addressed:

DA-3, DA-5, DC-1, DC-2, DC-3, DD-1,
DG-4

3. Experiment Title

Effects of Microgravity on the Reproductive Biology of a Novel Vertebrate Model: A Self-Fertilizing, Hermaphroditic Fish (Rivulus marmoratus).

4. Purpose/Hypothesis

Microgravity adversely affects the reproductive system of adult Rivulus marmoratus by lowering performance or efficiency. It may also modify the sex (gender) phenotypes of progeny which have developed in orbit.

5. Scientific Rationale/Rationale for Microgravity

This species, the only known regularly self-fertilizing vertebrate, has an autonomous reproductive system: each individual fish is a complete reproductive unit, without the need for separate sexes, courtship, or mating. Thus, components of reproductive biology like fecundity, fertility, development, etc. can potentially be monitored in orbit without the need to carry along males for sperm, mate animals, or to perform in vitro fertilizations. In addition, its clonal reproductive system means that genetically identical (and homozygous) organisms are available for comparative experiments. The bifunctional reproductive system of the species provides yet another potentially sensitive assay for the effects of microgravity. Its regulation is complex, subtle, and is already known to fail under some circumstances: temperature extremes or senescence can cause some hermaphrodites to sex invert to males. It may be disrupted by microgravity.

6. Approach

A. Experimental Duration: 90 days (plus post-flight).

B. General Approach

Description:

Genetically identical and age-matched adult hermaphrodites will be individually bred under both 0-g and 1-g (ground) conditions. The size of the ova they emit and their fecundity and fertility (general measures of female- & male-specific functioning, respectively) will be monitored. Zygotes will be reared, and their mortality, duration of embryonic development and primary sex phenotype will also be monitored. The comparison will be continued postflight to check for long term effects.

C. Number and Type of Specimen:

Approx. 30 adult hermaphrodites will be included in each group. The primary experiment will be with a Floridian clone that has shown no detectable sex inversion in the laboratory under any environmental conditions. The experiment can be readily extended to include a clone from Belize that has a relatively high rate of sex inversion to males.

D. Measurements/Sample

- Preflight: Rear appropriate numbers of isogenic hermaphrodites. Monitor fecundity and fertility of each specimen.
- In-flight: Collect ova every 48hrs, measure diameter & assess viability. Store developing ova in 30% seawater & monitor every 48 to 72 hrs. Assess mortality & developmental stage. Rear postlarvae & assess time to sexual maturity (appearance of first egg) & sex phenotype. Retain adult hermaphrodites & progeny for postflight comparisons.
- Postflight: Continue breeding adult hermaphrodites for at least 60 days postflight. Rear all progeny, including those born in-flight, & assess their reproductive performance relative to age-matched progeny of isogenic ground-based hermaphrodites.

E. Specific Sample Analysis:

- In-flight: See above.
- Postflight: Compare control, preflight, in-flight and postflight data by nested ANOVA and other statistical procedures.

F. Experimental Controls:

- In-flight: None
- Ground: Age-matched set of 30 genetically identical hermaphrodites, housed and fed identically to in-flight animals; their progeny.

7. Resource/Equipment Requirement: (On ground and in flight)

- In-flight: Breeding containers with plastic screen false bottoms for housing/breeding individual fish (4" dia. "finger bowls" or equivalent). Supplies of sea water and live food (brine shrimp) or freeze-fried equivalent (acceptance of latter by adult fish requires preflight training). Plastic petri dishes for holding and observing embryos and small plastic aquaria for rearing juveniles. Dissecting microscope with camera and ocular micrometer.
- Ground:

Experiment Identification Code: DC-i(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Dr. Konrad Dabrowski

2021 Coffey Road
Columbus, OH 43210

Critical Question(s) Addressed:

DB-3, DC-4, DB-2, DG-4

614-292-4555

3. Experiment Title

Development of in vivo Techniques to Study the Role of Ascorbic Acid (Vitamin C) in Ossification at Microgravity.

4. Purpose/Hypothesis

The teleost fish and primates (human) alike are missing vitamin C synthesis and as a result of calcification processes in cartilage matrix they may differ in composition of collagen and proteoglycans. It is believed that due to a post-translational changes in collagen helical structure, animals and humans with subclinical deficiency of vitamin C, suffer from deteriorated properties of their skeleton. Vitamin C is needed for the hydroxylation reactions that result in a right-handed superhelix of some 1000 peptide group molecules of collagen. In order to gather new information concerning a role of vitamin C in growth and ossification of cartilage/bone, a study is proposed with a teleost fish model.

5. Scientific Rationale/Rationale for Microgravity

The hydroxylation reaction, which vitamin C promotes in the synthesis of collagen, might be severely disturbed in a microgravity environment. To minimize this impact and associated pathologies, we hypothesize that the requirement for ascorbic acid needs to be significantly increased in comparison to the controls in the gravitational environment. Thus, changes in the concentration, structure and composition (hydroxylated amino acid) would be expected to affect the process of calcification. Our results will demonstrate the response of teleost fish models, which mimics well scurvy-prone mammals (and human), in respect to the effect of ascorbate status on cartilage and bone metabolism.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Juvenile catfish (Ictalurus punctatus) will be kept at microgravity or 1-g centrifuge. Fish will be offered a diet devoid of vitamin C or a diet supplemented with 500 ppm ascorbyl monophosphate.

C. Number and Type of Specimen:

400 newly hatched channel catfish of an individual weight 20 mg from one pair of parents.

D. Measurements/Sample

Preflight: Total fish analysis for ascorbic acid, collagen and proteoglycans. Purification of proteoglycans and analysis of hydroxyproline and hydroxylysine.

In-flight: Feeding animals formulated diets and taking samples of 5 fish from each treatment at weekly intervals and store at -80° C.

Postflight: Fish weight and mortality will be analyzed.

E. Specific Sample Analysis:

In-flight: 1-g controls.

Postflight: Perform and analyze electrophoretically purified collagens and proteoglycans. Analyze concentrations of individual amino acids and ascorbic acid compartmentalization in fish tissues.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Parallel 1-g controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Completely equipped biochemical laboratory for protein purification, electrophoresis and amino acid analysis (HPLC) is required. Structural protein analysis (proteoglycans) will be considered.

Ground:

Experiment Identification Code: DC-j(2) D = development; C = parturition/postnatal growth

1. Discipline: Developmental Biology

2. Author(s): Muriel D. Ross, Ph.D.

NASA Ames Research Center
Mail Stop 239-11
Moffett Field, CA 94035-1000

Critical Question(s) Addressed:

DC-4, DB-2, DG-4, DG-3, DG-2, DG-1,
DE-2

3. Experiment Title

Neural Development and Adaptation in a Microgravity Environment.

4. Purpose/Hypothesis

Tadpoles raised entirely in microgravity will fail to show significant adaptive changes in utricular macular synapses until the animals metamorphose into the amphibian stage. Centrifuged tadpoles, in contrast, will begin to show synaptic pruning between 5 & 7 days postnatally and will undergo further changes during metamorphosis in keeping with the presence of a gravitational field. Micro-g amphibians returned to earth will adapt within a period of 2-4 days.

5. Scientific Rationale/Rationale for Microgravity

Because the tadpole habitat is a water environment, it must be using light and other cues (air) to establish orientation: the water around it is exerting equal pressure and buoyancy effects experienced on earth do not apply. Neither is a polarized gravitational field of any possible influence. Under these conditions, the synapses cannot be pruned because the necessary interaction with gravity is absent. Micro-g centrifuged and ground control amphibians will exhibit greater changes in synapses because of the interaction between a polarized gravitational and genetic regulation. Upon return to earth, the micro-g amphibians will still be able to adapt to 1-g because they are first generation samples and have not been selected for a micro-g environment. However, expect some atypical vestibular-oculomotor responses upon landing and until adaption is complete.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Frogs will be stripped of eggs in space and the eggs will be fertilized there. Fertilized eggs will be divided form centrifuged and micro-g environments. Samples can be collected along the way, periodically, for ultrastructural study. Suggest days 6-8, 25,50,75 (immediately before metamorphosis is complete); day 1, day 4, and postlanding for amphibians. Ground controls will be matched. Also, testing of various kinds to learn whether swimming appears normal, and eye coordination is normal, and other organs should be examined besides the utricular maculas on what the tadpoles use for orientation. The importance of a centrifuge and age-matched samples, if differences are observed, these can be attributed to the effects of a lack of gravitational influences and not to radiation in space or age of examples.

C. Number and Type of Specimen:

A minimum of 8 samples should be collected for each time noted. The tadpoles should be fixed whole but a method to permit penetration of fixative would have to be worked out.

D. Measurements/Sample

Preflight: None

In-flight: 6-8, 25,50,75 (tadpole); days 1 and 4, amphibian.

Postflight: Day of landing, 4 days, 10 days.

E. Specific Sample Analysis:

In-flight: Samples collected, not analyzed (analyzed on the ground). Other tests TBD.

Postflight: Samples collected and analyzed by TEM, other methods as desired.

F. Experimental Controls:

In-flight: On 1-g centrifuge.

Ground: Matched to flight fertilization.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Centrifuge, habitats for tadpoles and amphibians; food storage; refrigerator for storage of chemicals; GPWS.

Ground:

Experiment Identification Code: DD-a(2) D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

2. Author(s): Dr. Suzanne Edmands and Dr. Ronald S. Burton

Marine Biology Research Division
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093-0202

Critical Question(s) Addressed:

DE-2, DF-2, DA-7, DC-1, DD-1, DG-5

3. Experiment Title

Metabolic Consequences of Multiple Generation Exposure to Microgravity.

4. Purpose/Hypothesis

Exposure to microgravity should decrease the rate of oxygen consumption for a given oxygen concentration. This may impair the synthesis or assembly of functional enzyme complexes such as cytochrome c oxidase (CO), resulting in reduced enzyme activity. Multiple generation of exposure to microgravity may then select for increased CO activity.

5. Scientific Rationale/Rationale for Microgravity

Previous work on cell cultures has shown reduced CO activity under low oxygen conditions, apparently due to a decrease in the synthesis or assembly of the mitochondrially translated subunits of cytochrome c oxidase. Microgravity conditions could mimic low oxygen conditions by altering the diffusion and convection of oxygen. Organisms which could survive and reproduce for multiple generations under such conditions would be under strong selection for increased CO activity.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Adult copepods will be allowed to mate and reproduce until day 15. All adults will then be removed and frozen. On days 30 & 60 all adults will again be removed and frozen. On day 90 half of the adults will be frozen; the remaining adults and juveniles will be returned to Earth alive. Cytochrome oxidase activity, measured by a standard spectrophotometric method and standardized by total protein, will be assessed postflight. Copepods returned alive will be assessed for "normalcy" (lifespan, larval survival, sex ratio, swimming and mating behavior etc.) and a subset of the survivors will be assessed for CO activity after they've acclimated to ambient gravity conditions.

C. Number and Type of Specimen:

Start with 100 adult male and 100 adult female copepods (Tigriopus californicus) for both experimental and control cultures.

D. Measurements/Sample

Preflight: Select and sex animals.

In-flight: Remove and freeze (-196° C) all adults on days 15, 30, & 60, and half of the adults on day 90. Return remaining animals to Earth alive.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Test live animals for "normalcy". Allow a subset of the live animals to acclimate to ambient gravity conditions for 48 hrs before freezing. Assess CO activity in all frozen samples.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Identical experiment at 1-g.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: -196° C storage freezer.

Ground:

Experiment Identification Code: DD-b(2) D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

2. Author(s): Dr. Clive L. Greenstock

AECL, Radiation Biology & Health Physics Branch
Chalk River, ON Canada K0J 1J0

Critical Question(s) Addressed:

DA-7, DB-4, DD-1, DG-5

3. Experiment Title

Microgravity and Space Radiation Effects on Stress Response in Insects.

4. Purpose/Hypothesis

To determine whether the conditions of space flight alter the biological response to oxidative stress in insects.

5. Scientific Rationale/Rationale for Microgravity

Any environmental perturbation can trigger biological changes that enable organisms to adapt and continue to flourish. Microgravity and/or space radiation will be tested for their ability to induce genetic and epi-genetic changes to combat oxidative stress. In particular, the level and persistence of gene expression and gene product associated with DNA damage (c-fos, cyclins, topo II, DNA Polymerase, GADD 45, MT, SOD, vits C & E, etc.) will be determined.

6. Approach

A. Experimental Duration: 1-90 days.

B. General Approach

Description:

Insects will be mated and allowed to reproduce throughout the flight. After the flight, the surviving adults and their offspring will be analyzed, utilizing standard protein and other biochemical and immunological tests.

C. Number and Type of Specimen:

At least 20 male and 20 female fruit flies (*Drosophila melanogaster*).

D. Measurements/Sample

Preflight: Sample assembly, control measurements.

In-flight: Minimal.

Postflight: Return samples to the lab for biochemical and immunological tests.

E. Specific Sample Analysis:

In-flight: None

Postflight: Measure total protein and induced gene products, and evaluate any effect of microgravity and/or space radiation.

F. Experimental Controls:

In-flight: None

Ground: Same as in-flight.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Sealed flasks to be installed in the GBF/ Insect Habitat.

Ground:

Experiment Identification Code: DD-c(2) D = developmental; D = multigenerational studies

1. Discipline: Developmental Biology

2. Author(s): Dr. William W.M. Steiner

Director, DOI, Pacific Islanders Science Center
Rm 406, Gilmore Hall -- UH
3050 Maile Way, Honolulu, HI 96822

Critical Question(s) Addressed:

DA-2, DA-3, DA-7, DB-4, DC-3, DD-1,
DE-3, DG-2, DG-5

3. Experiment Title

Boundary Effects at the Gene-Gravity Interface on Population Character, Metabolic Function, and Energy Flow Patterns in a Eukaryote.

4. Purpose/Hypothesis

To determine if differences in genetic background influence metabolic functioning and energy availability under different gravity potentials by monitoring genetic and biochemical changes through generational time using discrete populations subjected to microgravity differences.

5. Scientific Rationale/Rationale for Microgravity

Changes in the variable genetic base underlying energy production in natural populations of *Drosophila melanogaster* can sometimes be related to changes in environmental conditions. Defined responses have been found which relate closely to fitness differences usually measured as the relative ability to produce offspring. The effect of gravity on these changes has been assumed to be negligible, but gravity differences are theoretically influenced by electric fields. Internal, coordinated responses of cells, and genetic products such as enzyme mutations, are also known to be influenced by microelectric fields.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Eight genetic combinations will be set up in tube-cages in a row with each replicated three times. Each replicate will be contained in an automatic rotating chamber (ARC) set up under different microgravity regimes. Rotation of the chamber will occur every 18 days to realign each row of tube-cages with a fresh row for egg-laying to start the next discrete generation, relying in phototactic responses of the flies for attraction to the new egg-laying media. Each generation (e.g. row of tube-cages) will be removed and frozen for return to earth for genetic and biochemical analyses and after 5 generations the experiment will be terminated. Population data can be obtained from frozen individuals of each generation for study of microgravity effects on demographic effects and fitness traits.

C. Number and Type of Specimen:

20 mated pairs of known genotype will be placed in each of the initial 24 tube-cages (8x3 replications) in the three ARCs. Thereafter mating and egg-laying will be allowed to proceed at random. The broad genetic, physiological, biological, and ecological knowledge which exists for *Drosophila melanogaster* makes it the eukaryote of choice.

D. Measurements/Sample

Preflight: Prepare genetic strains for the experiment. Place 20 mated pairs of *D. melanogaster* into each of 24 tube-cages for loading into the ARCs. Repeat for each replication (3 total in-flight and 3 total ground based).

In-flight: Limited crew interaction: crew must remove tubes holding post-egg laying populations and freeze for postflight analysis.

Postflight: Count all members of frozen populations in each tube-cage; count number of eggs in remaining media; dissect 10 frozen females from each tube to determine reproductive condition.

E. Specific Sample Analysis:

In-flight: None

Postflight: Analyze each frozen individual using electrophoresis to determine genotypes at variable enzyme loci representing three different metabolic systems, correlate known biochemical parameters associated with each allele of each mutant type, correlate the same parameters to fitness traits associated with demographic and reproductive measurements, observe directional genetic changes for repeatability and comparison to controls; compare responses under different microgravity regimes. Repeatable but different responses from the controls constitutes evidence for gravitational effects.

F. Experimental Controls:

In-flight: As indicated above: 1-g constitutes a control class.

Ground: Experiments to be replicated in total on the ground. All genetic and biochemical tests and determination of fitness and demographic differences will be done on the ground.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: A total of 6, specially designed, interlocking ARCs containing tube-chambers for insect population studies capable of maintaining discrete generations are required; three for in-flight studies and three for groundbased controls. Design specifications are available.

Ground:

Experiment Identification Code: DE-a(2) D = developmental; E = behavior and circadian rhythms

1. Discipline: Developmental Biology

2. Author(s): Dr. Daniel Weber

Marine & Freshwater Biomedical Sciences Center
University of Wisconsin, 600 E. Greenfield Ave.,
Milwaukee, WI 53204

(414) 382-1726

Critical Question(s) Addressed:

DE-2, DG-4, DE-3, DC-1, DC-3, DB-1

3. Experiment Title

Effects of Microgravity on Reproductive Behavior in Fathead Minnows.

4. Purpose/Hypothesis

During nest preparation and maintenance, male fathead minnows orient to the underside of any submerged object; females also orient to that side during oviposition. Additionally, males must flip the female on her back during spermiation so that she will lay her eggs on the appropriate site. It is unknown to what degree gravitational cues are used to determine directions. Therefore, this study will examine the effects of microgravity conditions on breeding substrate orientation during all aspects of reproductive behavior in these fish.

5. Scientific Rationale/Rationale for Microgravity

There can be a variety of natural cues fish use to select their breeding sites; these cues may or may not be sex-specific. Although sex-steroid hormones are known to influence these behaviors, the role of gravity as a cue for the fish to select specific sides of an object for breeding is unknown. Proper orientation to a breeding site is critical for reproductive success; such orientation has already been shown to be disrupted by environmental contaminants. A disoriented male may prepare nest sites that are not acceptable for egg laying, e.g. exposed to predators. Females whose orientation has been disrupted may not lay her eggs in appropriate places. Males who have lost their sense of up and down may be unable to flip the female in the proper position for egg laying. An additional use of this study will be to understand potential effects of microgravity on human orientation abilities.

6. Approach

A. Experimental Duration: 30-60 days.

B. General Approach

Description:

Male and female fish will be placed in specially designed breeding chambers (breeding substrates attached to top, bottom or side). Behaviors/development of secondary sexual characteristics will be monitored by video recording. Daily egg counts will be recorded by photography. Post-flight data collection will involve histological analyses of gonads, % hatching and video tape analyses.

C. Number and Type of Specimen:

75 male/female fathead.

D. Measurements/Sample

Preflight: Breeding sets will be placed into aquaria 1 week before flight to acclimate fish to surroundings. Fish will be feed high-protein diet to maintain peak physiological condition. Photoperiod will be set at 16L:8D.

In-flight: Each day, video recordings of fish behavior will occur from time of lights (LO) to LO + 2 hr. Male-female behaviors at each substrate will be monitored for 10 minutes in random fashion. Each day, photographs of substrates will be taken to evaluate egg production & sites where eggs are oviposited. Video+ photographs of male/female

Postflight: secondary sex characteristics.

Postflight: Fish will be fixed in formalin, including any hatched fry and prepared for histological analyses. All photographs and video recordings will be cataloged as to day, direction of substrate, egg counts and development of secondary sex characteristics.

E. Specific Sample Analysis:

In-flight: None

Postflight: Histological analyses on gonadal development. Egg counts: number, rate of development, percent hatching. Video tapes: time spent in each behavioral category, orientation to specific sites of substrate, secondary sex characteristics, parental care of eggs, specifically orientation toward eggs.

F. Experimental Controls:

In-flight: None

Ground: Identical experiment will be conducted.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Specially designed aquaria with pumps (no bubbling of air), CCD video, Vita-Lite Full spectrum lights, temperature regulator, carbon filters, recirculating dechlorinated water, food, PVC breeding substrates. Within each aquarium, substrates will be attached to different walls to simulate different orientations.

Ground:

Experiment Identification Code: DE-b(2) D = developmental; E = behavior and circadian rhythms

1. Discipline: Developmental Biology

2. Author(s): Dr. Daniel Weber

Marine & Freshwater Biomedical Sciences Center
University of Wisconsin, 600 E. Greenfield Ave.,
Milwaukee, WI 53204

(414) 382-1726

Critical Question(s) Addressed:

DE-2, DG-4, DC-4, DG-1, DG-2

3. Experiment Title

Effects of Microgravity on Orientation Response During Schooling Behavior in Zebrafish.

4. Purpose/Hypothesis

Schooling behavior is a common activity in many fish species and has strong adaptive value. Because individual movements within the school depend upon the lateral line neurons which are sensitive to changes in hydrostatic pressure, it is possible that under conditions of microgravity, the neuronal response time may be altered enough to change schooling behavior in response to adverse stimuli.

5. Scientific Rationale/Rationale for Microgravity

Fish, and some amphibians, possess a peripheral nerve structure known as the lateral line. Located along the sides of the fish, the neurons in this system are sensitive to changes in hydrostatic pressure, thus allowing them to assess velocity and orientation of and distance to nearby objects, e.g. members of its school, potential predators or source of adverse stimuli. Changes in hydrostatic pressure are known to have unequal effects on different life stages of zebrafish; effects on schooling behavior are unknown. Should microgravity have an effect on either hydrostatic pressure, lateral line neuron function, or both then it is possible that the ability of a fish to maintain its position within the school could be affected. Schools change size and shape in response to stimuli; it is these characteristics that can be used to assess the role microgravity may have on lateral line control of specific fish behaviors.

6. Approach

A. Experimental Duration: 14 days.

B. General Approach

Description:

In specially designed 2l aquaria, zebrafish (fry, juvenile and adult) will acclimate for one week. During the second week, a pulsed sound stimulus will be applied to the aquarium every 5 minutes for 1 hr. video recordings will be made for 3-dimensional analyses of group reaction. Ultrasonic transducers will be placed on aquarium to monitor activity level.

C. Number and Type of Specimen:

75 zebrafish of 3 age-classes; total = 225

D. Measurements/Sample

Preflight: Separate fish into 3 age-classes (fry, juvenile and adult) and acclimate to aquarium conditions for one week.

In-flight: Video recording of school response to pulsed sound stimulus plus computer collection of activity levels as determined by ultrasonic transducer recordings. These tests will be done 1x/day for 7 days after 1 wk acclimation. Pumps off during testing.

Postflight: Repeat test after one week acclimation to determine if behavior returns to normal.

E. Specific Sample Analysis:

In-flight: None

Postflight: Video recordings: frame-by-frame analysis for school volume changes, time to recover from stimulus, nearest neighbor distances, orientation of each fish pre and post-stimulus, velocity of individual movement. Computer analysis of activity levels.

F. Experimental Controls:

In-flight: None

Ground: Identical experiment will be conducted.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Specially designed aquaria with pumps (no bubbling of air), CCD video, Vita-Lite full spectrum lights, temperature regulator, carbon filters, recirculating dechlorinated water, food, ultrasonic transducers on aquarium walls, sound production device, computer for image analyses.

Ground:

Experiment Identification Code: DE-c(2) D = developmental; E = behavior and circadian rhythms

1. Discipline: Developmental Biology

2. Author(s): Dr. Paul W. Webb

School of Natural Resources & Environment,
University of Michigan
Ann Arbor, MI 48109-1115

313-764-2550 Fax: 313-936-2195

Critical Question(s) Addressed:

DE-2, DC-4, DG-4, DG-3

3. Experiment Title

Stability of Fish with Different Body and Fin Forms Under Low Gravity.

4. Purpose/Hypothesis

Spiny-rayed fish will be better able to control body posture than soft-rayed fish at low gravity.

5. Scientific Rationale/Rationale for Microgravity

Neutral buoyancy among aquatic organisms provides a freedom to move through three-dimensional aquatic space similar to the zero gravity experiences of humans in space. Fish are characterized by a distribution of median and paired fins around the center of mass that generate thrust forces to control posture and rotate the body in any direction, a solution similar to that of humans maneuvering in low-gravity space. I predict that the less-derived body plan characteristic of soft-rayed fish will result in a reduced ability to control body posture during routine tasks compared with the more derived pattern seen among acanthopterygians.

6. Approach

A. Experimental Duration: 30 days.

B. General Approach

Description:

Medaka or zebra danio, representative of typical soft-rayed fishes, and angelfish representative of highly maneuverable spiny-rayed fishes will be maintained in a 1-g and 0-g environment. Fish will be held in a tubular aquarium divided into two parts by a variable width slit (Webb LaLiberte & Schrank, Environ. Biol. Fish. in press). Fish will be induced to pass through the slits with horizontal and vertical orientations until the minimum widths are determined. This simple task measures the relative ability to control posture, while comparisons between the 1- and 0-g situations will show the consequences of low gravity on posture control. Fish movements in the chambers and in passing through the slits will be recorded (or broadcast for ground-based recording) on video-tape for ground-based analysis.

C. Number and Type of Specimen:

10 medaka or zebra danios (soft-rayed fishes), mass approximately 1 gm (3 to 5 cm in total length) and 10 angelfish (spiney-rayed fishes) of similar mass will be used. These fish have been studied for many years in my laboratory and are known to be robust and easy to care for. The soft-rayed species have already been used in 0-g situations.

D. Measurements/Sample

Preflight: acclimate fish of each species to separate chambers.

In-flight: Video tape fish swimming in the chambers and induced to swim through slits of various sizes.

Postflight: None

E. Specific Sample Analysis:

In-flight: Record minimum vertical and horizontal slit widths negotiable according to established criteria based on time limits and backing away behavior.

Postflight: Analysis of video tape to compare.

F. Experimental Controls:

In-flight: Record transit of fish through slits in variable-gravity centrifuge at 1-g.

Ground: Replicate experiment on the ground at the same time as the 0-g experiments are performed.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Two fish chambers, to be fabricated by the investigators, limited variable gravity centrifuge use, video camera and tape, air supply for fish in chambers, fish food.

Ground: Same hardware for ground controls.

Experiment Identification Code: DE-d(2) D = developmental; E = behavior and circadian rhythms

1. Discipline: Developmental Biology

2. Author(s): Dr. Gordon M. Burghardt

Department of Psychology
University of Tennessee
Knoxville, TN 37996-0900

Critical Question(s) Addressed:

DE-2, DB-3, DC-1

3. Experiment Title

Prenatal Development of Coordinated Behavior in Microgravity.

4. Purpose/Hypothesis

Microgravity will cause changes in the embryonic development of sensory, motor, and neural circuits underlying the typically accurate ability of snapping turtles to locate, orient towards, and capture prey. Effects on hatching behavior will also be studied.

5. Scientific Rationale/Rationale for Microgravity

Snapping turtle eggs are almost perfectly round and exposed embryos respond to tactile cues with well oriented movements. Microgravity should both reduce and alter the tactile and other sensory cues normally sensed by the developing embryo during its "spontaneous" prenatal movements. This should be manifested in more poorly developed locomotor and feeding behavior, compared to 1g controls when tested at 1g.

6. Approach

A. Experimental Duration: To hatching and 2 days beyond. Duration depends on the age of eggs when flight begins, but should be no more than 60 days.

B. General Approach

Description:

Fertile turtle eggs (*Chelydra serpentina*) will be incubated and hatching videotaped. Their locomotor, righting, defensive, and prey capture abilities will be compared to normally incubated controls.

C. Number and Type of Specimen:

16 eggs from 2 clutches (8 each) on station and 16 eggs from the same two clutches will be incubated at 1g on Earth.

D. Measurements/Sample

Preflight: Record egg size and mass. Monitor incubation temperature.

In-flight: Videotape hatching and locomotor behavior.

Postflight: Test for terrestrial and aquatic locomotion, defensive striking (snaps), and orientation (visual, chemical) towards food and accuracy in snapping at it.

E. Specific Sample Analysis:

In-flight: Record hatching and locomotor behavior.

Postflight: Tests of controls and experiment. Test for terrestrial and aquatic locomotion, defensive striking (snaps), and orientation (visual, chemical) towards food and accuracy in snapping at it.

F. Experimental Controls:

In-flight: 1-g centrifuge controls if centrifuge available, otherwise none.

Ground: Controls will parallel flight experiments.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Videotape for hatching and locomotor behavior.

Ground: Videotape for hatching and locomotor behavior.

Experiment Identification Code: DG-a(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Richard N. Winn, Ph.D.

Center for Applied Isotope Studies
University of Georgia
120 Riverbend Road Athens, GA 30606

706-542-1395

Critical Question(s) Addressed:

DG-5, DB-4, DG-1, DG-4

3. Experiment Title

Detection of Radiation-Induced Mutations Using Transgenic Fish.

4. Purpose/Hypothesis

Chromosomally-inserted target genes in fish embryos will integrate radiation-induced mutations over time, and will be reliable indicators of in vivo mutagenesis during space flights.

5. Scientific Rationale/Rationale for Microgravity

The potential health risks of exposure to space radiation remains incompletely known. Transgenic animal models that carry well-defined mutation reporter genes offer new approaches not otherwise available in detecting mutations induced during space travel and may provide more realistic assessment of genotoxicity, and the mechanisms of DNA damage and repair.

6. Approach

A. Experimental Duration: 1- 16 days.

B. General Approach

Description:

Transgenic fish embryos (unhatched) will be exposed to space radiation under several regimens (e.g. exposure duration, degree of shielding). Embryos will be maintained with minimal flight crew involvement in chambers for the exposure duration and frozen at designated times in flight and at flight termination. Radiation mini-dosimeters attached to chambers will quantitate radiation exposure. Mutations in the target gene will be analyzed in on-ground studies.

C. Number and Type of Specimen:

Transgenic fish embryos (Fundulus heteroclitus or Oryzias latipes; 1-1.8 mm diameter) will be held in replicated (3) exposure chambers ~30 embryos/chamber with five time treatments.

D. Measurements/Sample

Preflight: Prepare embryos (in vitro fertilize eggs).

In-flight: Set up embryo culture chambers in various radiation exposure regimens. Remove selected embryo cultures and freeze (-70oC) at time points.

Postflight: None

E. Specific Sample Analysis:

In-flight: None

Postflight: Recovery of mutation marker from fish DNA and characterization of mutation frequencies.

F. Experimental Controls:

In-flight: Time 0 control.

Ground: Controls will parallel in-flight experiments under several radiation exposure regimens.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: a. Minimal in-flight requirements- -70dC freezer.
b. Hardware requirements for ground- Same as for in-flight hardware.

Ground:

Experiment Identification Code: DG-b(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Dr. Eberhard Horn

University of Ulm, Dept of Neurology, Section of
Neurophysiology
Albert-Einstein-Allee 11
D - 89081 Ulm, Germany
xx49-731-502 4565 Fax: xx49-7344-3630

Critical Question(s) Addressed:

DA-1, DC-4, DG-1, DG-4, DB-2, DG-2,
DG-3

3. Experiment Title

Development of the Static Vestibulo-ocular Reflex During Long-term Exposure of Fish Youngsters and/or Amphibian Tadpoles to Microgravity.

4. Purpose/Hypothesis

It will be tested whether and at which period of life, gravity deprivation affects the development of the static vestibulo-ocular reflex (VOR) irreversibly. It is postulated that the effect of gravity deprivation in the static VOR depends (1) on the onset and duration of u-g during development, and (2) on the developmental velocity of the individual animal. It is expected (1) that an onset even of a few-days lasting ug-exposure during the early development is more effective than a later onset even if this period is considerably longer, and (2) that the static VOR of developmentally retarded youngsters is more affected by gravity deprivation than that of their normally developing siblings.

5. Scientific Rationale/Rationale for Microgravity

Experiments have shown that altered gravitational conditions affect the development of the static VOR in the fish Oreochromis mossambicus and the amphibian Xenopus laevis. The changes were reversible within 1-3 weeks but persisted longer in developmentally retarded youngsters. The concept of the "sensitive period" considers irreversible changes in the efficiency of a sensory system. To apply this concept to the development of the vestibular system, animals have to be exposed to ug during different periods of their life. Fishes and amphibians are suitable model animals because (1) the recording of their VOR offers an excellent method to test the efficiency of a "deprived" vestibular system, and (2) the individual developmental velocity, which differs tremendously in siblings, allows a reliable classification into fast and slowly growing animals.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

The fish Oreochromis and/or the amphibian Xenopus will develop from the egg stage under ug-and 1g-condition. Samples will be exposed only once to a short- or long-lasting period of ug, which will begin in the different samples at different periods of life. After ug-termination, the youngsters will be tested in-flight for their VOR threshold and, under 1g-in-flight condition, for their VOR gain. These animals will be fixed for histological examinations thereafter. Special emphasis will be given to the comparison of the VOR threshold and gain in developmentally retarded youngsters with respect to their normally developing siblings.

C. Number and Type of Specimen:

At the onset of the experiments, about 500 fish and/or tadpole youngsters. The total number of samples will be 12, composed of 3 different durations of the ug-periods and 4 different developmental stages at which ug begins ($3 \times 4 = 12$). Each behavioral test will be performed with 10 to 15 youngsters. The same number will also be fixed.

D. Measurements/Sample

Preflight: Egg fertilization. Packing of samples into transport containers.

In-flight: Starting and terminating ug-exposure in the different samples. Video-recordings of the static VOR at several times after ug-termination using a specific equipment. Fixation of half of the animals after the first testing, the rest will stay alive and will be transferred to in-flight 1g-condition.

Postflight: Recording of the VOR at several times to determine 1g-readaptation of the samples with the longest exposure to ug as well as the persistence of the VOR deficits.

E. Specific Sample Analysis:

In-flight: None, because only recordings and fixation activities are necessary.

Postflight: Analyses of the in- and post-flight video-recordings of the VOR. Histological analyses with special reference to the development of central nuclei and their anatomical connections within the vestibular to oculomotor pathways, using anterograd and retrograde and retrograde tracer methods.

F. Experimental Controls:

In-flight: Half of the samples for 1g control; same recording and fixation procedure as in the ug-exposed samples.

Ground: Same procedure as in space station with 2 hrs time delay.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Specific device for the VOR recordings which has to be adapted from the earth-used model to space implementation. Holding facility for aquatic animals with light from above and 1g-reference centrifuge. Freezer for stowage of samples.

Ground:

Experiment Identification Code: DG-c(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): A.P. Farrell

Biological Sciences
Simon Fraser University
Burnaby, B.C. V5A 1S6 Canada

604-291-3647

Critical Question(s) Addressed:

DC-4, DG-3, DG-4, DA-7, DC-1

3. Experiment Title

Gravity Effects in the Expression of the Ryanodine Receptor on the Developing Heart.

4. Purpose/Hypothesis

Parturition in mammals and hence exposure to gravity is associated with a marked expression of the cardiac ryanodine receptor. The ryanodine receptor, located in the sarcoplasmic reticulum, is central to the control of cardiac excitation-contraction coupling.

5. Scientific Rationale/Rationale for Microgravity

It is expected that comparative studies of aquatic, semi-aquatic and terrestrial species under varying gravitational conditions will reveal the extent to which gravity rather than vascular resistance contributes to the expression of this important cellular protein.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Animals will be bred and juveniles raised in 0-g, 1-g and 2-g conditions. Heart samples will be taken at three developmental stages for analysis of ryanodine receptor density. Hearts will be homogenized and receptor densities analyzed within a ^3H -ligand for ryanodine receptors.

C. Number and Type of Specimen:

Breeding pairs representative of fish, amphibian and mammals(Fundulus, Xenopus and rat?) to yield 15 offspring. A minimum of 5 offspring will be sampled at three different developmental stages.

D. Measurements/Sample

Preflight: Prepare breeding stock; examine effect of freezing on ryanodine receptor assay.

In-flight: Care for animal stock; sampling of hearts; homogenization of hearts; freezing of homogenate samples.

Postflight: Radioligand binding studies.

E. Specific Sample Analysis:

In-flight: Observations of reproductive success and viability of offspring.

Postflight: Standard radioligand assays for ryanodine receptors on frozen heart homogenates.

F. Experimental Controls:

In-flight: 1-g controls.

Ground: Parallel 1-g controls.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Rearing chambers/aquaria for experimental animals; materials for sampling, homogenizing and freezing (-196°C) heart tissues in-flight; Liquid scintillation counter and apparatus for radioligand assays post-flight.

Ground:

Experiment Identification Code: DG-d(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Keith C. Cheng, M.D,Ph.D.

Div. of Experimental Pathology
Penn State College of Medicine
500 University Dr.
Hershey, PA 17033
717-531-5635

Critical Question(s) Addressed:

DG-5, DF-3, DA-7,DG-4, DG-1

3. Experiment Title

The Effect of Gravity on Tissue-specific Patterns of p53 Expression during Zebrafish Embryogenesis.

4. Purpose/Hypothesis

The long-term goal is the coordination and development of practical means to assess the mutagenic and carcinogenic risks of long-term exposure to radiation in space. We propose to examine patterns of p53 expression in zebrafish embryos at various stages of embryogenesis by whole-mount in situ hybridization. Microgravity and/or radiation may affect this expression. The results will have implications for assessing cellular risk and gestation in space.

5. Scientific Rationale/Rationale for Microgravity

Space Station personnel are exposed to microgravity as well as increased and variable doses of several types of radiation, some specific to space travel. The mutagenic and carcinogenic risk associated with this increased exposure remains to be established. These experiments are inspired by knowledge of the central role of p53 as a checkpoint protein during the cell cycle and as the most commonly mutated gene in human cancer. Since p53's expression may be intimately related to proliferation and differentiation of various cell types, its tissue-specific patterns of expression may have implications for tissue-specific risk to cancer.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Using a recently cloned p53 gene from the zebrafish, transcriptional patterns in embryos can be determined by whole-mount in situ hybridization. Embryos spawned in space will be collected at various stages of development and fixed for in situ hybridization for examination of tissue-specific patterns of p53 expression. Radiation doses will be determined and measurements will be done in collaboration with established Health Physics investigators. This work should be coordinated with that of other investigators interested in developmental patterns of tissue-specific gene expression.

C. Number and Type of Specimen:

On the order of 200 embryos will be generated either on the ground and/or in space for collection at various times of development.

D. Measurements/Sample

Preflight: Obtain complete sequence of p53 clone and establish tissue-specific patterns of expression of zebrafish embryos during normal embryogenesis and physical and chemical genotoxic stresses. Sibling embryos will be generated as controls.

In-flight: The embryos will be grown and fixed after varying times during embryogenesis for whole-mount in situ hybridization in the ground to determine tissue-specific patterns of transcription.

Postflight: Whole-mount in situ hybridization of fixed zebrafish embryos, sectioning and photography of stained embryos.

E. Specific Sample Analysis:

In-flight: Inspection fixation of embryos at different stages of development.

Postflight: See Measurements/Sampling Handling.

F. Experimental Controls:

In-flight: Embryos and fish shielded from radiation; embryos and fish at centrifugal 0.5 and 1g

Ground: Siblings of embryos.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Aquaculture facilities, incubators, shaker, dissecting and normal microscopes, dissecting tools, camera.

Ground:

Experiment Identification Code: DG-e(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Keith C. Cheng, M.D,Ph.D.

Div. of Experimental Pathology
Penn State College of Medicine
500 University Dr.
Hershey, PA 17033
717-531-5635

Critical Question(s) Addressed:

DG-5, DF-3, DB-4, DG-4

3. Experiment Title

Development of in vivo Methods to Monitor Potential Mutagenic and Carcinogenic Risk Associated with Space Radiation.

4. Purpose/Hypothesis

The long-term goal is the coordination and development of practical means to assess the mutagenic and carcinogenic risks of long-term exposure to radiation in space. The initial thrust, focusing upon mutagenesis, will be the development and ground-based testing of three zebrafish models for detection mutation. Coordination with radiation measurements will be done in collaboration with established Health Physics investigators, and development of comparable medaka models will be done in collaboration with established workers in that field.

5. Scientific Rationale/Rationale for Microgravity

Space station personnel are likely to be exposed to increased and variable doses of several types of radiation, some specific to space travel. The mutagenic and carcinogenic risk associated with this increased exposure remains to be established. Monitoring of this risk must be ongoing due to the variable nature of radiation in proportion to solar flares, changes in position of the space station with respect to radiation belts, and the relative positions of the space station, earth and radiation sources. The development of a practical, and yet comprehensive and coordinated, set of experiments to assess mutagenic and carcinogenic risk is important to maximize information obtained per units of weight, space, and cost.

6. Approach

A. Experimental Duration: Continuous (monitoring tool).

B. General Approach

Description:

Zebrafish embryos heterozygous for recessive pigmentation mutations such as *gol-1*, will detect loss-of-function mutations in the remaining wild-type allele by the presence of mosaic eyes in which clones of cells have lost the normal pigmentation associated with pigmented retinal epithelial cells. Each embryo will be screened by dissecting/traditional microscope for non-pigmented cells among about 1100 in monolayer in the eyes in which individual nonpigmented cells will be detectable. Additional information will be gained by generation and use of zebrafish transgenic for multiple copies of a lambda phage *lacI* shuttle vector which can be packaged and plated on *E. coli* lawns for both mutation rate and mutational spectrum determination. Correlation between results of these experiments and those mutagenic risk due to and/or weightlessness.

C. Number and Type of Specimen:

On the order of 1000 golden and/or albino heterozygous embryos will be examined for mosaic eyes. DNA from 10-20 adult transgenic fish will be packaged in lambda phage using extracts, and plated for determination of mutation rate and spectrum upon return.

D. Measurements/Sample

Preflight: Zebrafish lines transgenic for one or more proven candidate shuttle vectors will be established, tested for low background, & tested for utility of detecting radiation risk.

In-flight: The transgenic strain found to be the best in the preflight experiments will be used in space. For the in-space experiments, we currently estimate that about 1000 golden and/or albino heterozygous embryos, 1000 transgenic embryos, & 10-20 adult transgenic fish will be generated.

Postflight: In-flight: The embryos will be scored for mosaic eyes & fixed for confirmation on the ground postflight, DNA from the transgenic embryos & adult fish may be prepared in part at various times in flight.

Postflight: Confirmation of mosaic eye frequency. Packaging & plating of lambda phage. Scoring of mutation frequency, purification of mutants & sequencing of mutations.

E. Specific Sample Analysis:

In-flight: Microscopic inspection of embryo eyes for mosaic eyes.

Postflight: Confirmation of mosaic eye frequency. Packaging & plating of lambda phage. Scoring of mutation frequency, purification of mutants & sequencing of mutations.

F. Experimental Controls:

In-flight: Embryos and fish shielded from radiation; embryos and fish at centrifugal 0.5 and 1-g.

Ground: Siblings of embryos and adult fish.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Aquaculture facilities, in-flight radiation shielding, incubators, dissecting and normal microscope, camera, sequencing facilities.

Ground:

Experiment Identification Code: DG-f(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Keith C. Cheng, M.D,Ph.D.

Div. of Experimental Pathology
Penn State College of Medicine
500 University Dr.
Hershey, PA 17033
717-531-5635

Critical Question(s) Addressed:

DA-7, DF-3, DG-5, DG-1, DG-4

3. Experiment Title

The Effect of Gravity and Radiation on Tissue-specific Patterns of hsp70 Gene Expression During Zebrafish Embryogenesis.

4. Purpose/Hypothesis

The long-term goal is the coordination and development of practical means to assess physiologic and teratogenic risks of long-term exposure to radiation and microgravity in space. As an indicator of cellular stress, tissue-specific patterns of hsp70 gene expression in zebrafish embryos will be examined at various stages of embryogenesis by whole-mount in situ hybridization. Microgravity and/or radiation may affect this expression. The results will have implications for monitoring cellular stress in space and for in-space gestation.

5. Scientific Rationale/Rationale for Microgravity

Space station personnel are exposed to microgravity as well as increased and variable doses of several types of radiation, some specific to space travel. Cellular stress due to these factors may occur in a tissue-specific manner. The hsp70 gene, whose importance is revealed by a very high level of evolutionary conservation from bacteria to man, is expressed as a result of heat and other forms of stress. It is of interest, therefore, to determine whether stress caused by microgravity and/or radiation affects patterns of tissue-specific expression of hsp70 at different stages of vertebrate development.

6. Approach

A. Experimental Duration: Variable, estimated 7 days.

B. General Approach

Description:

Using a recently cloned hsp70 gene from the zebrafish, transcriptional patterns in embryos spawned in space will be collected at various stages of development and fixed for in situ hybridization for examination of tissue-specific patterns of hsp70 gene expression. This work will be coordinated with that of other investigators interested in developmental patterns of tissue-specific gene expression to most efficiently use the limited space available on the space station and time of the space station scientists.

C. Number and Type of Specimen:

On the order of 200 embryos will be generated either on the ground and/or in space for collection at various times of development.

D. Measurements/Sample

Preflight: Obtain complete sequence of hsp70 genomic and cDNA clones and establish tissue-specific pattern of expression of zebrafish embryos during normal embryogenesis and after heat shock. Sibling embryos will be generated as controls.

In-flight: Embryos will be examined and fixed at different stages of development.

Postflight: Whole-mount in situ hybridization of fixed zebrafish embryos, sectioning and photography of stained embryos.

E. Specific Sample Analysis:

In-flight: Inspection fixation of embryos at different stages of development.

Postflight: See: Measurements/Sample Handling (above).

F. Experimental Controls:

In-flight: Embryos shielded from radiation; embryos at centrifugal 0.5 and 1g.

Ground: Siblings of embryos submitted to no shock and to different degrees of heat shock.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Aquaculture facilities, incubators, shaker, dissecting and normal microscopes, dissecting tools, camera.

Ground:

Experiment Identification Code: DG-g(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Dr. Frieda B. Taub

School of Fisheries
University of Washington
Box 355100
Seattle, WA 98195

Critical Question(s) Addressed:

DG-4, DB-2, DB-3, DC-2, DC-4

3. Experiment Title

Effects of Microgravity and Exercise on Bone and Muscle Morphology in Aquatic Vertebrates (Fish).

4. Purpose/Hypothesis

Fish, being accustomed to the buoyancy of water will not lose muscle mass when exposed to long term microgravity. Exercise will increase muscle and bone strength and morphology under microgravity, but only to the same degree as at 1-g. This will contrast with terrestrial vertebrates (humans, rodents) which do exhibit significant bone loss and muscle weakness.

5. Scientific Rationale/Rationale for Microgravity

The effects of microgravity are being tested on basic physiological processes to determine if aquatic organisms have advantages of maintaining health under microgravity conditions.

6. Approach

A. Experimental Duration: 90 days.

B. General Approach

Description:

Microgravity condition (1) microgravity with gentle water flow (just adequate to supply O₂) (2) microgravity with rapid water flow to provide exercise. Using young Medaka (fish), place 6 immatures into each Aquatic Habitat. Midway in the experiment (if possible) remove 3 for preservation for muscle and bone morphology studies, At the end of the experiment test for stamina, bone and muscle morphology studies. At the end of the experiment test for stamina, bone and muscle morphology.

C. Number and Type of Specimen:

30-60 Medaka fish.

D. Measurements/Sample

Preflight: Growth, general health, stamina.

In-flight: Swimming behavior (video).

Postflight: Stamina (ability to swim against a current); bone & muscle morphology.

E. Specific Sample Analysis:

In-flight: None

Postflight: None

F. Experimental Controls:

In-flight: Ground controls at two rates of water flow; survival, growth.

Ground: Stamina, general health.

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight: Minimum on orbit requirements.

Ground: Hardware requirements for ground.

Experiment Identification Code: DG-h(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Dr. Bernard C. Wentworth

Department of Poultry Science
University of Wisconsin
Madison, WI 53706-1284

Critical Question(s) Addressed:

3. Experiment Title

Feeding Day-old, Young, and Adult Quail in Microgravity.

4. Purpose/Hypothesis

Newly-hatched, young and adult quail can be efficiently fed and watered in microgravity using a complete quail ration containing 70% water in a gelatin mold.

5. Scientific Rationale/Rationale for Microgravity

The respiratory structure and function along with the hardness of avian beaks prevents the sucking mechanism needed to obtain water from the nipple type waterers in microgravity. This type of watering system is entirely dependent upon gravity for the avian species. Formulation of a gel-mold quail ration (GMQR) containing 5% gelatin, 70% water and 25% complete quail ration, could be designed to feed day-old, young and adult quail in microgravity as the needs arise.

6. Approach

A. Experimental Duration: 90 days (from hatching to hatching - 4 separate hatches).

B. General Approach

Description:

GROUND BASED STUDY: Initial experiments will be conducted in gravity to raise newly hatched quail to successful reproduction with GMQR fed ad libitum on a daily basis. Hatch mate controls will be fed the same complete quail ration ad libitum by the conventional feeding system and using nipple waterers.

IN MICROGRAVITY: This procedure could be adapted to newly-hatched, young or adult quail in microgravity. The GMQR and container could be made to fit on the outside of the incubator/or animal enclosure model, within easy eyesight and reach of the enclosed birds. A fresh GMQR container can be easily adfixed to the incubator or animal enclosure module each day.

C. Number and Type of Specimen:

Sixty wild type quail will be hatched, banded, individually weighed and randomly placed into two groups of 30 birds each.

D. Measurements/Sample

Preflight: The GMQR will be prepared in advance of the experiment and irradiated to preserve its quality without refrigeration. It will be prepared in containers designed to provide access to the ration while keeping evaporation to a minimum. Group 1 will be fed the
In-flight: GMQR and group 2 will be the controls, as previously described. The birds will be weighed weekly. At two weeks of age, 24 males and 24 females per group will be randomly selected (on the basis of feather colorations typical of the sex) to be maintained on experiment for the remainder of the experimental period. At four weeks
Postflight: of age, the complete quail starter ration will be replaced with the complete quail breeder ration, both in the GMQR and in the control groups. Starting at 42 days of age, eggs will be collected and set at weekly intervals for four weeks. All hatched birds will be raised for 2 weeks on the respective diets for viability comparisons.

E. Specific Sample Analysis:

In-flight: Weekly weight gain, feed consumption, feed conversion, days to puberty, number of eggs laid by adult females, egg weights, shell quality, semen quality, fertility, hatchability and viability of hatch, will be the major data collected for management
Postflight: comparison.

F. Experimental Controls:

In-flight:

Ground:

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

Experiment Identification Code: DG-i(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Dr. Bernard C. Wentworth

Department of Poultry Science
University of Wisconsin
Madison, WI 53706-1284

Critical Question(s) Addressed:

3. Experiment Title

Long Term Storage of Eggs Before Incubation.

4. Purpose/Hypothesis

We hypothesize that a major cause of the reduced hatchability associated with the interval between laying and start of incubation is dehydration.

5. Scientific Rationale/Rationale for Microgravity

There is a delay associated with egg collection packaging, transport in orbit flight prior to space station docking and also some additional delay before the start of incubation. This interval may be as long as 13 days. We believe that if the eggs are stored at 13C in PBS pH 7.0 containing Tylosin that the hatchability will be improved.

6. Approach

A. Experimental Duration: 1-17 days.

B. General Approach

Description:

Initial work on this project will be done on the ground. There will be 48 fertile eggs subjected to four treatments.

1. Twenty-four eggs will be held dry at 13C.

2. Twenty-four eggs will be stored at 13C in PBS pH 7.0 containing TBD ppm Tylosin.

Another group of 48 will be handled for the same length of time under the same condition and will serve as the synchronous ground controls.

C. Number and Type of Specimen:

There will be 48 eggs used in flight and another 48 eggs used as ground controls.

D. Measurements/Sample

Preflight: Described in General approach "a"

In-flight: Hatchability will be the major end pointy. Either this PI or other PIs could use the hatched quail in an add-on experiment.

Postflight: Analysis of hatching data.

E. Specific Sample Analysis:

In-flight:

Postflight:

F. Experimental Controls:

In-flight:

Ground:

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

Experiment Identification Code: DG-j(2) D = developmental; G = other

1. Discipline: Developmental Biology

2. Author(s): Dr. Bernard C. Wentworth

Department of Poultry Science
University of Wisconsin
Madison, WI 53706-1284

Critical Question(s) Addressed:

3. Experiment Title

Management of Day-Old Quail in Microgravity.

4. Purpose/Hypothesis

Newly hatched, quail chicks can be assisted early in life by magnetic feet that will keep them lightly attached to the metal surface.

5. Scientific Rationale/Rationale for Microgravity

The initial feeding behavior is facilitated by experience. Consequently the inability of the newly hatched precocious bird (quail) to orient itself makes feeding difficult.

6. Approach

A. Experimental Duration: 40 days (From hatching to puberty).

B. General Approach

Description:

Quail will be hatched in microgravity. Determination will be made to decide if they can be managed better with magnetic feet as compared to a tethered body suit in microgravity. The ground controls of body suit and free moving chicks will be the reference for microgravity.

C. Number and Type of Specimen:

Forty-eight eggs incubated for 13 days in the ground laboratory will be transferred to incubator and hatched in a microgravity environment.

D. Measurements/Sample

Preflight: One-half of the newly hatched quail will have their feet coated with magnetic iron oxide (A formulation is too long to detail here) and evaluated by weight gain, and behavior as to the success of this method of management. The other half will be placed in fitted

In-flight: body suits and held in position for feeding.

Postflight: The ground controls will serve as the hatch reference weight, but the weight gain, feed conversion and days to puberty will be the major data for management comparison.

E. Specific Sample Analysis:

In-flight: The amount of food consumed. With the appropriate design of the feed this can be estimated closely by volume.

Postflight: Immediate post-flight the long bones will be scanned to obtain comparative data on bone strength of quail with magnetic feet, with comparison to body suit and ground controls. The feed consumption, weight gain, feed conversion and age at puberty will provide additional comparisons. Also, a post-flight evaluation of reproductive performance for 60 days would provide treatment impact.

F. Experimental Controls:

In-flight: The 48 ground controls have been described in 6A, B, C, D and E. They are critical to meaningful data comparisons.

Ground:

7. Resource/Equipment Requirement: (On ground and in flight)

In-flight:

Ground:

APPENDIX A

CRITICAL QUESTIONS AND GBF EXPERIMENT MATRICES

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Critical Questions

The enclosed critical questions are taken from the NASA Space Biology Program's Cell, Developmental, and Plant Biology Discipline Plans.

An individual experiment may address more than one critical question and more than one critical question sub-category. All the critical questions addressed by each experiment are identified in the reference experiment descriptions. The bolded critical question entry in each reference experiment description is the question that is most directly addressed by the experiment.

Use of GBF Experiment Matrices

Reference experiments are listed across the top of each matrix in a three letter shorthand notation, e.g. CA-a.

Critical Questions are identified along the side of each matrix using two letters and a number, e.g. CA-1.

The first two letters in both codes describe the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The final letter/number makes the notation unique by indicating where the experiment/question is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments. Critical Question CA-1 is the first of the Cell Biology Gravity Sensing/Response critical questions.

The column labeled "Total" is the sum of the checks along each row and gives the total number of reference experiments which address each critical question.

To locate specific experiments, refer to the Experiment Index, page 3-1. The Experiment Index lists the experiments in order, according to their experiment identification codes. To facilitate location, the experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page.

Critical Question Sub-Categories

- (C) Cell Biology
- (CA) Gravity Sensing/Response
- (CB) Transduction/Response
- (CC) Other
- (D) Developmental Biology
- (DA) Early Developmental Events
- (DB) Later Developmental Events
- (DC) Parturition/Postnatal Growth
- (DD) Multigenerational Studies
- (DE) Behavior/Circadian Rhythms
- (DF) Aging
- (DG) Other
- (P) Plant Biology
- (PA) Gravity Perception/Transduction/Response
- (PB) Reproduction/Development
- (PC) Metabolism/Transport
- (PD) Communities/Microecology

Cell Biology Critical Questions

Cell Biology (C):

CA. Gravity Sensing/Response:

1. Are single cells capable of sensing and responding to changes in gravity, or are only multicellular systems capable? Do single cells "sense" gravity directly (intracellularly) or indirectly (environmentally-mediated effect)?
2. If single cells sense changes in gravity directly, what are the intracellular structural/functional mechanisms that are sensitive to gravity perturbation? Is the cytoskeleton organization of cells disturbed by gravity perturbation? How does the cell's cytoskeleton, outer membrane and nuclear envelope/nuclear matrix react to altered gravity, as a three-dimensional continuum of perception and structural integrity?
3. If single cells are too small to detect changes in the gravitational field directly, what are the environmental changes responsible for the cells' response? Is the cessation of microconvection currents at microgravity responsible?
4. Do single cells sense alterations in gravity directly, in which cells are part of a gravisensing organ, or indirectly, in which the cells detect indirect consequences of the presence or absence of inertial acceleration?
5. How do the following modifying factors affect gravity "sensing" at the cell level: cell size, cellular dynamics; changes in cell shape; prokaryotic versus eukaryotic cells adaptive versus non-adaptive cells; circadian rhythms?
6. If multicellular systems are necessary for gravity sensing, how is this effected? What cellular structures and processes that extend across several cells might be involved? What aspects of cell-cell communication are affected? Would the requirements for cellular interaction/assembly increase sensitivity to indirect or environmentally mediated effects (e.g., reduction of cell-cell and cell-surface contact by dispersion of cells in microgravity)?

CB. Transduction/Response:

1. What are the mechanisms involved in the transduction of the stimulus of altered gravitational force to a cellular response? By what pathways is the perception of altered gravity relayed intracellularly and/or extracellularly?
2. Research indicates that resting/native cells are not measurably affected by changes in gravity. What is responsible for the difference in responsiveness between resting and active cells?
3. How are the following cell functions influenced by gravity and/or affected by microgravity: the expression and regulation of genetic information; cell division; cell differentiation; signal transduction, including signal-membrane interactions, membrane-effector interaction, and signal-effector linkage; membrane dynamics; intracellular transport; secretion; alternate pathway regulation; and cell-to-cell communication? The importance of selecting cells and cell lines that can provide interpretable results bearing on precise questions cannot be overemphasized.

4. How does the gravity stimulus affect cellular responses following the binding of specific growth factors to their cognate membrane receptors--as an independent variable or a quantifier? What are the contributions of the cytoskeleton, the intracellular pathways of chemically mediated signal transfer, and the nuclear envelope/nuclear matrix to functional response?
5. How will altered gravitational fields and vectors change the information content of the three-dimensional microenvironment of the cells (stroma and matrix connections)? How does microgravity affect these signals under both homeostasis and challenge?
6. How are cell-cell and cell-surface contacts in multicellular systems affected by microgravity?
7. When do gravitational effects appear? Are there differences between responses that occur as a direct consequence of acute exposure to microgravity and responses at a later time, that may reflect the operation of compensatory mechanisms?
8. How long can single cells cope with changes in gravitational force without adverse results? Do these effects persist after return to unit gravity?

CC. Other:

1. How is the effect of gravity (and microgravity) on cells influenced by magnetic fields and radiation?
2. How can gravity be used as a research tool in perturbing cell structure/function in the absence of other effectors?
3. To take the best advantage of flight opportunities, how can life science experiments on cellular systems be automated in protocol, remote manipulation, and data retrieval and analysis?

GBF CELL BIOLOGY EXPERIMENT CRITICAL QUESTIONS

[illegible]

Developmental Biology Critical Questions

Developmental Biology (D):

DA. Early Developmental Events (Gravity effects on fertilization through organogenesis):

1. What will be the result of gravity-induced dys-synchrony (temporal or hormonal) during embryonic development?
2. Which developmental mechanisms have evolved to be dependent on the 1-g gravity field and vector?
3. What events in gametogenesis and early germ cell maturation are gravity sensitive, and how can these results relate to the proliferation and differentiation of other individual cell types?
4. Can altered gravities affect fertilization, and do these results indicate more general mechanisms of membrane alteration in individual cells?
5. Which responses are transmitted maternally and which are intrinsic to the developing embryo?
6. What are the results of altered gravity fields on the axis polarity and symmetries of the zygote?
7. Are there gravity effects that can terminate in changes of gene activation?

DB. Later Developmental Events (Gravity effects on organogenesis through the development of anatomical structures):

1. Which organ systems are dependent on the 1-g gravity field and vector.
2. How does gravity affect organogenesis and the development of anatomical structures?
3. What structural and morphometric alterations will occur in the extracellular matrix, the connective tissue, and the musculoskeletal systems? How will this result in altered differentiation of cells, and in changed tissue composition?
4. How do specific organs and tissues respond developmentally to altered gravity, as demonstrated by the expression of selected target genes in transgenic mice with pre-determined genetic makeups?

DC. Parturition/Postnatal Growth:

1. Will hatching or parturition occur normally?
2. In the period of rapid-natal growth, which systems are the most sensitive to altered gravity perturbations?
3. What are the effects of gravity, in concert particularly with life in closed ecosystems, on sexual maturation?
4. Are the gravity sensitive systems (i.e., vestibular, proprioceptive, cardiovascular, musculoskeletal) of young and adult animals similarly sensitive to this stimulus in ontogeny?

DD. Multigenerational Studies:

1. Does the developmental ontogeny of animals raised through more than one life cycle under a changed gravity field differ from the 1-g classical pattern? Does this altered pattern reside in the genome, or is it relayed from hormonal and stromal interactions?

DE. Behavior/Circadian Rhythms:

1. At what developmental stage can we observe perturbations of circadian rhythms, both temporally and with respect to differentiation state?
2. How will gravitational fields, particularly microgravity, disturb the precise coordination and postural control required in mating?
3. How will parent-young interactions be altered in the space environment?
4. What will be the effects on lactation, suckling and related parent-young bonding mechanisms?

DF. Aging:

1. How does gravity produce responses in cultured cells that mimic those seen in chronologically aged cells, those isolated from accelerated aging syndromes, and senescent cells *in vitro*?
2. Which de-limiters of lifespan have relevance to gravitational effects?
3. How do neoplasms common to chronological aging relate to limitations of cell lifespan and susceptibility to abnormal growth regulation under altered gravitational fields?

DG. Other:

1. Are there critical windows of susceptibility for developmental processes, or is development affected in a gradient?
2. If gravity related effects exist, can they be reversed in the short or long term?
3. Considering development as a series of stages or phases, beginning with pattern specification and progressing through differentiation, how will gravity affect selected phases in animals that represent different species and phyla?
4. Will aquatic animals perceive and respond to gravity as do their terrestrial counterparts? Those animals which pursue different life states in both environments may be particularly valuable for study.
5. What are the joint effects of radiation and microgravity?

[illegible]

[illegible]

Plant Biology Critical Questions

Plant Biology (P):

PA. Gravity Perception/Transduction/Response

1. What are the mechanisms that underlie gravity perception?
2. What are the sequential events in gravity transduction and response?
3. How do single cells sense gravity?
4. What are the thresholds required for gravity to have an effect?
5. What changes in the routes of perception, transduction and response occur in microgravity?
6. What are the differences, if any, between species and their tissues in their perception and responses to gravity?

PB. Reproduction/Development

1. Can plants successfully reproduce through more than one generation in space?
2. Is chromosomal integrity and behavior during cell division affected in microgravity?
3. Is cell, tissue, or organ differentiation affected in microgravity?
4. What effect does microgravity have on embryogenesis and the ensuing stages of the life cycle of plants from maturity to flowering and senescence?
5. Are microgravity-grown tissues and organs competent?
6. Are the growth rates of higher plants or single cells affected by microgravity?
7. How do plants adapt to microgravity?
8. Are there unique interactions between space radiation (or other environmental factors) and microgravity that affect the development of biological systems in space?

PC. Metabolism/Transport

1. Are anabolic and catabolic pathways and the photosynthetic apparatus and pathway altered in microgravity?
2. What effect does microgravity have on the synthesis of storage and support polymers?
3. What are the effects of the space environment on membranes and transport during uptake and secretion?
4. Are pathways for nutrient absorption altered in microgravity?

5. What are the effects of the space environment on long distance transport of water and on transpiration?
6. What are the mechanisms by which transport systems are polarized in plants grown in space?

PD. Communities/Microecology

GBF PLANT BIOLOGY EXPERIMENT CRITICAL QUESTIONS

[illegible]

APPENDIX B

GBF SCIENCE WORKING GROUP MEMBERS

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• SWG Project/Program Scientists	B-2
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GBF SWG Project/Program Scientists

Dr. Charles Wade - SWG Chair
GBF Project Scientist
NASA Ames Research Center
Mail Stop 239-11
Moffett Field, California 94035-1000
Tele: (415) 604-3943
NASAMail address: CEWADE
E-mail: Charlie_Wade@qmgate.arc.nasa.gov

Dr. William Knott - SWG Co-Chair
GBF Deputy Project Scientist
John F. Kennedy Space Center
National Aeronautics and Space Administration
Mail Code MD-RES, Hangar L
Kennedy Space Center, FL 32899-0001
Tele: (407) 853-5142
NASAMail address: WMKNOTT

Dr. Thora Halstead
GBF Program Scientist
National Aeronautics and Space Administration
Life and Biomedical Sciences Division/Code UL
300 E. Street, SW
Washington, D. C. 20546
Tele: (202) 358-2148
NASAMail address: THALSTEAD

GBF Cell Biology SWG Members

Dr. Terry Johnson - Discipline Chair
Division of Biology
Kansas State University, Ackert Hall
Manhattan, KS 66506
Tele: (913) 532-6705
E-mail: terryJ@KSUVM.KSU.EDU

Dr. Joseph F. Albright
Chief, Basic Immunology Branch
Division of Allergy, Immunology and Transplantation
National Institute of Allergy and Infectious Diseases
Solar Building, Room 4A25
Bethesda, MD 20882
Tele: (301) 496-7551

Dr. Bruce Umminger
Director, Division of Integrative Biology and Neurosciences
National Science Foundation
1800 G Street, NW
Washington, D.C. 20550
Tele: (202) 357-7905
E-mail: bumminge@NSF (BITNET)
 bumminge@nsf.gov (INTERNET)

Dr. Peggy Whitson
Biomedical Operations and Research Branch
NASA Johnson Space Center
SD 4, Bldg. 37
Houston, TX 77058
Tele: (713) 483-7046

Dr. William P. Wiesmann
Division of Surgery Combat Trauma & Casualty Research
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100
Tele: (202) 576-3791

GBF Developmental Biology SWG Members

Dr. Patricia "Scotti" Hester - Discipline Chair
Dept. of Animal Sciences
Purdue University
1026 Poultry Science Bldg.
West Lafayette, IN 47907-1026
Tele: (317) 494-8019
E-mail: PATRICIA_HESTER@ACN.PURDUE.EDU

Dr. Dora "Holly" Hayes
Livestock Insects Laboratory
Livestock and Poultry Science Institute
Room 120, Bldg. 307 BARC-E
10300 Baltimore Ave.
Beltsville, MD 20705-2350
Tele: (301) 504-8474

Dr. Martin Morton
Department of Biology
Occidental College
Los Angeles, CA 90041
Tele: (213) 259-2674

Dr. Carey Phillips
Department of Biology
Bowdoin College
Brunswick, ME 04011
Tele: (207) 725-3573
E-mail: CPhillip@bowdoin.edu

Dr. Gerald Schatten
Department of Molecular Biology and Zoology
University of Wisconsin
1117 W. Johnson Street
Madison, WI 53706
Tele: (608) 262-5746
E-mail: SCHATTEN@MACC.WISC.edu

Dr. Richard Wassersug
Dalhousie University
Department of Anatomy & Neurobiology
Sir Charles Tupper Bldg.
Halifax, Nova Scotia
Canada B3H 4H7
Tele: (902) 494-2244
E-mail: tadpole@ac.dal.ca

GBF Plant Biology SWG Members

Dr. Norman Lewis - Discipline Chair
Institute of Biological Chemistry
Washington State University
Pullman, WA 99164-6340
Tele: (509) 335-2682
E-mail: LEWISN@WSUVM1.csc.wsu.edu

Dr. Arnold J. Bloom
Department of Vegetable Crops
University of California
Davis, CA 95616
Tele: (916) 752-1743
E-mail: AJBloom@UCDavis.edu
AJBloom@UCDavis (BITNET)

Dr. Gerald Deitzer
Department of Horticulture
University of Maryland
College Park, MD 20742
Tele: (301) 405-4335
E-mail: GD3@umail.umd.edu

Dr. Lewis Feldman
Department of Plant Biology
University of California
Berkeley, CA 94720
Tele: (510) 642-9877
E-mail: feldman@insect.berkeley.edu

Dr. Fred Sack
Department of Botany
Ohio State University
1735 Neil Avenue
Columbus OH 43210
Tele: (614) 292-0896
E-mail: sack.1@osu.edu

Dr. Abraham D. Krikorian
Department of Biochemistry and Cell Biology
State University of New York
Stony Brook, NY 11794
Tele: (516) 632-8568

APPENDIX C

GBF SOLICITATION PACKAGE

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Note: The SWG list is not included in this appendix since it is provided in Appendix B.

Dear Colleague:

Space life science research programs will constitute a major portion of the research that will be performed on the Space Station. This offers the scientific community a unique opportunity for studying the affect of gravity on the development and physiology of living organisms. The Gravitational Biology Facility will provide specimen habitats and laboratory equipment to support Cell Biology, Developmental Biology, and Plant Biology research on board the Space Station. The Facility will be developed in an evolutionary manner beginning with items that support "simple" experiments, from the standpoint of equipment development and crew time requirements. The development process will gradually evolve toward the support of more complex experiments that require the development of more sophisticated hardware and increased crew time.

The Gravitational Biology Facility, being developed by NASA Ames Research Center under the supervision of the NASA Life Sciences Division in the Office of Space Science and Applications, consists of generic hardware items that will be used in multiple experiments on orbit. In order to identify the hardware items, a set of reference experiments is being created for which the equipment needs will be identified. The resulting list of equipment will be analyzed to determine the final list of Gravitational Biology Facility hardware to be developed for the Space Station.

To ensure that the equipment suite developed supports a broad range of research, the Gravitational Biology Facility Project is soliciting reference experiments from you and other members of the science community. The reference experiments are one page summaries that describe the hypothesis, scientific rationale, general approach, and equipment needs of each experiment. The equipment needs should be specified in terms of generally available laboratory equipment. A reference experiment template and other information is enclosed to aid you in the development of your reference experiments. Please work with the Gravitational Biology Facility Science Working Group member who provided you with this package (or his/her designee, if any) in order to develop one or more reference experiments. Your completed reference experiments should be submitted to your contact on the Science Working Group no later than April 6.

The reference experiments will be accumulated and published as a NASA reference document that will be made available to the public. Participation in this activity is at the discretion of each researcher and there is no obligation to or from the National Aeronautics and Space Administration. This is in not a NASA Research Announcement (NRA) or an Announcement of Opportunity (AO) or an agreement to fund future research.

Thank you for your time and effort. The contribution of your expertise will greatly enhance the quality and functionality of the Gravitational Biology Facility.

Sincerely,

Charles E. Wade, Ph.D., Gravitational Biology Facility Project Scientist

Kellie A. McKeown, Gravitational Biology Facility Project Manager

* Versions of this letter were released on February 21, and June 5, 1992

DESCRIPTION OF ENCLOSURES

1. Science Working Group: A list of the Gravitational Biology Facility Science Working Group members is enclosed. These individuals are a resource for providing additional information regarding the Gravitational Biology Facility and the reference experiment development process. Your reference experiments can also be submitted to them and they can assist you in developing the information required in the reference experiments.
2. Experiment Categories: Candidate reference experiments should focus on the critical space biology research issues for Cell, Developmental, and Plant Biology as indicated on the Experiment Categories enclosure.
3. Reference Experiment Template: Reference experiments should be written in the format of the enclosed "Reference Experiment Template" and returned no later than June 29, 1992.
4. Example Reference Experiments: Three example reference experiments are enclosed to show the desired detail and content of the reference experiments. Each example represents a different phase of the Space Station as discussed below. The Cell Biology example is applicable for a short duration, Spacelab class mission (Phase 1); the Developmental Biology example is applicable for an extended duration, unmanned flight (Phase 2); and the Plant Biology example is appropriate for the fully-manned phase of the Space Station (Phase 3).

SPACE STATION BACKGROUND INFORMATION

The Space Station will be fully developed and permanently manned in 1999. However, prior to the final configuration, gravitational biology investigations can take advantage of early flight opportunities which will have limited crew support. The reference experiments should be designed to fit into one of the three mission scenarios described below. Specific emphasis should be placed on experiments that can be flown during the early flight opportunities (Phase 1 and 2).

Phase 1 (Spacelab class missions) - This phase provides flight opportunities similar to Spacelab missions with a total flight time of 16 days (approximately 11 days can be used for science payload operations on orbit). The Space Shuttle will be docked at the Space Station and members of the astronaut crew will be available to perform basic experiment procedures including specimen sampling and fixation for ground-based analysis.

Phase 2 (Spacelab class missions with extended unmanned durations) - This phase is similar to the Spacelab Class Missions except the science payload can be left unattended on board Space Station for 30 - 180 days after the Space Shuttle returns to Earth. This mission requires autonomous science experiments that do not require on-orbit crew manipulation.

Phase 3 (Space Station missions) - This is the most sophisticated stage of the Space Station in which the crew will be permanently on orbit and experiment durations are not limited. Research facility capabilities available on orbit will be more diverse and complex. Specimen handling and analysis will be able to be performed on-orbit.

Discipline:

A. Cell Biology

1. Cell Activation - (Gravity perception; interactions with cell and environment)
 - Cell-cell interactions, cell-surface interactions
 - Activation sequences
 - Agonist-ligand interactions
2. Transduction - (Intracellular events)
 - Second messenger pathways
 - Genetic control
3. Growth, Differentiation, Dedifferentiation
 - Protein synthesis
 - Post translational modifications
4. Metabolism
 - Mitochondria
 - Aerobic
 - Anerobic
5. Functional Capacity
 - Muscle contraction
 - Phagocytosis
 - Antibody production
6. Stress Response
 - Ubiquitin pathway
 - Heat-shock proteins
 - Acclimation and compensation mechanisms
7. Other Considerations
 - Eukaryotic vs. prokaryotic organisms

EXPERIMENT CATEGORIES

Discipline:

B. Developmental Biology

1. Role of gravity in developmental biology
 - Alterations in the developmental ontogeny of animals raised in space vs. animals raised in 1 g
 - Critical windows of susceptibility for developmental processes
 - Reversal of gravity related effects
 - Gravity-induced dys-synchrony during embryonic development
2. One g dependent developmental mechanisms
3. Gravity effects on early developmental events
 - Gametogenesis, early germ cell maturation
 - Fertilization
 - Embryonic axis determination
 - Zygotic gene activation
4. Effects on developmental phases across different species and phyla.
 - Aquatic vs. terrestrial animals
5. Stages of perturbations of biological rhythms
6. Organogenesis and development of anatomical structures
7. Structural and morphometric alterations
8. Gravity effects on behavior
 - Parent/offspring interactions
 - Sexual behavior
9. Sexual maturation
10. Reproductive systems
11. Effects of gravity on lifespan de-limiters
12. Joint effects of radiation and microgravity

EXPERIMENT CATEGORIES

Discipline:

C. Plant Biology

1. Gravity perception, transduction, and response mechanisms
 - Gravity perception mechanisms
 - Sequential events in gravity transduction and response
 - Gravity sensing by a single cell
 - Effective gravity thresholds
 - Changes in the routes of gravity perception, transduction, and response
 - Perception and response differences between species.
2. Reproduction and development processes
 - Reproduction through more than one generation
 - Cell division
 - Cell, tissue, and organ differentiation
 - Life cycle stages (embryogenesis through senescence)
 - Competency of microgravity grown tissues and organs
 - Growth rates
 - Adaptation to microgravity
 - Developmental effects due to unique interactions of space radiation and microgravity (or other environmental factors)
3. Metabolism, photosynthesis, and transport processes
 - Changes in photosynthetic apparatus, anabolic and catabolic pathways
 - Synthesis and storage of support polymers
 - Effect on membranes and transport during uptake and secretion
 - Nutrient absorption pathways
 - Long distance water transport and transpiration
 - Mechanisms for polarization of transport systems

Reference Experiment Template

1. Discipline

2. Author/Reference

(Responsible Science Working Group member: name

Author: name, address, phone number)

3. Experiment Title

4. Purpose/Hypothesis (~ 3 sentences)

5. Scientific Rationale/Rationale for Microgravity (~ 4 sentences)

6. Approach

A. FLIGHT/EXPERIMENT DURATION

Incorporate to one of the following:

Phase 1(beginning 1997)- 16 days (includes launch/landing and 13 days on-orbit). Similar to Spacelab flight, capabilities limited to very simple on-orbit analysis and preservation with most analysis on ground.

Phase 2 (1997-1999)- Shuttle docked for 9-13 days at beginning and end of mission with 30-180 days unmanned in between. Capabilities limited to very simple on-orbit analysis and preservation with most analysis on ground.

Phase 3 (beginning 1999)- Variable experiment duration. Starting with simple on-board analysis and evolving to more complex on-board analysis. Please state experiment duration for experiments during this phase.

B. GENERAL APPROACH DESCRIPTION: (~ 4 sentences)

C. NUMBER AND TYPE OF SPECIMEN:

D. MEASUREMENTS/SAMPLE HANDLING: (brief descriptions)

Preflight:

In-flight:

Postflight:

E. SPECIFIC SAMPLE ANALYSIS: (brief descriptions)

In-flight:

Postflight

F. EXPERIMENT CONTROLS: (brief descriptions)

In-flight:

Ground-based:

7. Hardware Requirements

A. MINIMUM ON ORBIT REQUIREMENTS

B. HARDWARE REQUIREMENTS FOR GROUND

8. Names, addresses, and phone numbers of other scientists interested in contributing:

EXAMPLE - Phase 1 Experiment

1. Discipline

Cell Biology

2. Author/Reference

Peggy A. Whitson, Ph.D. (GBF Science Working Group Member)
NASA/Johnson Space Center, Mail Code SD4
Houston, TX 77058
Phone: 713-483-7046

3. Experiment Title

Microgravity Effects on Cytoskeletal Components of Attachment-Dependent Cells and Suspension Cells

4. Purpose/Hypothesis

Microgravity-induced alterations in the cytoskeleton will differ in attachment-dependent vs. suspension cells.

5. Scientific Rationale/Rationale for Microgravity

Previous studies have suggested that microgravity altered microtubules of heart tissue and tradescantia microspores and that hypergravity enhanced phosphorylation of microtubule-associated protein. Therefore, a study examining the effects of microgravity on the transcription and phosphorylation characteristics of the cytoskeleton and its components will provide valuable knowledge of cellular level function in microgravity.

6. Approach

A. FLIGHT/EXPERIMENT DURATION

Phase 1 - 16 days

B. GENERAL APPROACH DESCRIPTION:

Cells will be cultured in-flight in both 0 g and 1 g conditions. Replicate cell samples will be taken daily from each of the cultures and will be frozen (-196 ° C). Messenger RNA transcription and phosphorylation of cytoskeletal and associated proteins will be assessed postflight.

C. NUMBER AND TYPE OF SPECIMEN:

A lymphocyte-derived and an endothelial cell line will be used. Approximately 12 duplicate samples from each experimental condition (4) would be expected (96 total samples).

D. MEASUREMENTS/SAMPLE HANDLING:

Preflight:	Prepare cell stocks
In-flight:	Set up cell cultures, remove daily samples from each culture and freeze.
Postflight:	None

E. SPECIFIC SAMPLE ANALYSIS:

In-flight:	None
Postflight:	mRNA isolation and quantification of tubulin, actin, vimentin; characterization of cytoskeletal associated protein phosphorylation

F. EXPERIMENT CONTROLS:

In-flight:	1 g controls
Ground-based:	Controls will parallel in-flight experiments in 1 g.

7. Hardware Requirements

A. MINIMUM ON ORBIT REQUIREMENTS

CO₂ cell incubator, 1 g CO₂ cell incubator, sterile sampling/set up environment (laminar flow hood), -196 ° C storage freezer.

B. HARDWARE REQUIREMENTS FOR GROUND

Same as flight hardware

EXAMPLE - Phase 2 Experiment

1. Discipline

Developmental Biology

2. Author/Reference

(Author: name, address, phone number)

Responsible Science Working Group member: name)

3. Experiment Title

Multigeneration Reproduction in Insects.

4. Purpose/Hypothesis

No organism has traversed a complete life cycle (egg-to-egg) during sustained altered gravity conditions. A study is needed to determine the effects of microgravity on the development and fecundity of late- generation organisms produced in microgravity.

5. Scientific Rationale/Rationale for Microgravity

Need to determine whether multigenerations can be obtained in microgravity and whether animals produced under these conditions experience any selection pressures (e.g. male, female) and whether late-generation organisms are normal compared to ground-based insects.

6. Approach

A. FLIGHT/EXPERIMENT DURATION:

Phase 2 - 90 days

B. GENERAL APPROACH DESCRIPTION:

Insects will be allowed to reproduce through multiple generations for at least 90 days (8 generations). This can be accomplished without crew involvement except at the end of the flight period when the habitat containing the last generation of insects and their prospective offspring will be sent to Earth for postflight analysis.

C. NUMBER AND TYPE OF SPECIMEN:

Start with 20 male and 30 female fruit flies (*Drosophila melanogaster*)

D. MEASUREMENTS/SAMPLE HANDLING:

Preflight:	None
In-flight:	None
Postflight:	Examine body weights, sex ratios, percent of eggs that develop, lifespan, and other physiological and biochemical parameters designed to determine "normalcy" of the last generation of insects in microgravity.

E. SPECIFIC SAMPLE ANALYSIS:

In-flight:	None
Postflight:	None

F. EXPERIMENT CONTROLS:

In-flight:	None
Ground-based:	Same as in-flight

7. Hardware Requirements

A. MINIMUM ON ORBIT REQUIREMENTS

Insect habitat (pressurized, temperature regulation, light, etc..)

B. HARDWARE REQUIREMENTS FOR GROUND

Same as in-flight for ground controls, dissecting microscope, small mass measuring device

EXAMPLE - Phase 3 Experiment

1. Discipline

Plant Physiology

2. Author/Reference

(Author: name, address, phone number

Responsible Science Working Group member: name)

3. Experiment Title

Role of Microgravity in Lignification

4. Purpose/Hypothesis

One structural response to gravitational stress or gravity load in woody plants on Earth is lignification. Plants grown in microgravity will not form lignin like their Earth-based counterparts.

5. Scientific Rationale/Rationale for Microgravity

To understand the role of gravity in control of development at the organ and whole plant level. To understand the role of gravity in regulating metabolic and cellular processes in plants.

6. Approach

A. EXPERIMENT DURATION:

Phase 3 - 90 days

B. GENERAL APPROACH DESCRIPTION:

Plant specimens will be maintained in zero-g and at various g levels on the variable gravity centrifuge. At various intervals, plants will be harvested, dissected and samples fixed for ground-based microscopic and enzymatic analyses.

C. NUMBER AND TYPE OF SPECIMEN:

Pine seeds, 48

D. MEASUREMENTS/SAMPLES:

Preflight: Chemically determine the amount of lignification in pine to determine morphological and anatomical distribution of lignin.

In-flight: Harvest and dissect specimen. Fix samples at various times.

Postflight: None.

E. SAMPLE ANALYSIS:

In-flight: UV absorption microscopy

Postflight: Electron microscopy and enzymatic analysis.

F. EXPERIMENT CONTROLS:

In-flight: Variable-gravity centrifuge controls.

Ground-based: Ground-based controls.

7. Resource Requirements

A. MINIMUM ON ORBIT HARDWARE REQUIREMENTS

Plant growth chamber, variable gravity centrifuge, tissue fixation equipment, dissection equipment, -70°C freezer, -196°C freezer, refrigerator, UV microscope

B. GROUND BASED HARDWARE REQUIREMENTS

Same as in-flight hardware for ground controls, electron microscope and related equipment

APPENDIX D

GBF HARDWARE CAPABILITIES LIST AND EXPERIMENT MATRICES

CONTENTS

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•	GBF Hardware Capabilities List from July 1992	D-2
•	GBF Hardware Capabilities Definitions as of July 1992	D-3
•	Hardware Capabilities Matrices	
-	Use of Matrices	D-6
-	Cell Biology Experiment Hardware Capabilities Matrix	D-7
-	Developmental Biology Experiment Hardware Capabilities Matrix	D-8
-	Plant Biology Experiment Hardware Capabilities Matrix	D-10

GBF HARDWARE CAPABILITIES LIST from JULY 1992

Habitats (Both 0g and 1g environments provided on-orbit)

Cell Culture Apparatus
Small Plant Habitat
Insect Habitat
Small Aquatic Habitat (Fresh Water)
Medium Aquatic Habitat (Fresh Water)
Avian Egg Incubator
Rodent Birthing Habitat
Small Aquatic Habitat (Salt Water)
Large Plant Habitat
Medium Aquatic Habitat (Salt Water)
Rodent Breeding Habitat
Avian Hatchling Habitat
Rodent Rearing Habitat
Rodent Weanling to Adult Habitat
Avian Adult Habitat
Large Aquatic Habitat

Multi-discipline Equipment

Data Storage
Fluid Handling Tools
Compound Microscope
Work Area
Dissection Equipment
Dissecting Microscope
Temperature Controlled Chamber
Temperature Controlled Laboratory Centrifuge
Radioisotope Handling Equipment
Digital Multimeter
Ion Selective Electrodes
Spectrophotometer
Mass Measurement Device
Electrophysiology Measuring Equipment
Micromanipulation Device
Luminometer

Preservation and Storage Equipment

Snap/Quick Freezer
-70°C Freezer
Chemical Fixation Capability
Refrigeration (4°C)
-196°C Freezer
-20°C Freezer
Freeze Dryer
Ambient Storage

Habitats (Both 0g and 1g environments provided on-orbit)

Cell Culture Apparatus - The cell culture apparatus includes capabilities for: a) Supporting prokaryotic and eukaryotic cells, and tissue cultures, b) Growing cells on a variety of surfaces, c) Manipulating cells and cell media (including sample collection and injection of experimental compounds, media supplements, drugs, fixatives, etc.) d) Combining gases (e.g. CO₂) with liquid media., e) Thorough mixing of fluids, f) Temperature control g) Sterile culturing, h) Automation and computer control to the extent possible, and i) Variable gravity conditions.

Small Plant Habitat - Plant growth chamber with approximately 500 cm² growing area. Provides environmental monitoring and control (temperature, gas levels, pH, humidity, conductivity, vibration); video monitoring; and data logging.

Insect Habitat - Habitat to maintain a variety of insects, including Drosophila, through multiple generations. Provides environmental control and video monitoring.

Small Aquatic Habitat (Fresh Water) - Multiple < 200 ml aquatic chambers that support small fresh water organisms. Provides environmental control (temperature, pH, gas levels, lighting), waste management and video monitoring. Optimally, this habitat will also accommodate salt water.

Medium Aquatic Habitat (Fresh Water) - 200 ml to 5 liter aquatic habitat that supports fresh water organisms. Provides environmental control (temperature, pH, gas levels, lighting), waste management, nutrient delivery, and video monitoring. Optimally this habitat will be combined with the Medium Salt Water Aquatic Habitat into a single Medium Aquatic Habitat.

Avian Egg Incubator - Environmentally controlled incubator (temperature, humidity) to maintain chicken and quail eggs. Auto fixation, and egg turning capability.

Rodent Birthing Habitat - Habitat to accommodate female rodents during pregnancy and birth. Provides food, water, waste management, environmental control, lighting, and video monitoring.

Small Aquatic Habitat (Salt Water) - Multiple < 200 ml salt water chambers that support small marine organisms. Provides environmental control (temperature, pH, gas levels, lighting), waste management, and video monitoring. Optimally, this habitat will also accommodate fresh water.

Large Plant Habitat - Centrifuge Facility Project Plant Habitat. Provides approximately 1300 cm² growing area environmental monitoring and control (temperature, gas levels, pH, humidity, conductivity, vibration); video monitoring; and data logging.

Medium Aquatic Habitat (Salt Water) - 200 ml to 5 liter salt water habitat that supports marine organisms. Provides environmental control (temperature, pH, gas levels, lighting), waste management, nutrient delivery, and video monitoring. Optimally this habitat will be combined with the Medium Fresh Water Aquatic Habitat into a single Medium Aquatic Habitat.

Rodent Breeding Habitat - Centrifuge Facility Project Rodent Habitat. Special habitat for breeding rodents. Provides food, water, waste management, environmental control, and video monitoring.

Avian Hatchling Habitat - Habitat to support newly hatched birds. Provides food, water, environmental control, and video monitoring.

Rodent Rearing Habitat - Special habitat which facilitates nursing of rodent pups in microgravity. Provides food, water, waste management, environmental control, lighting, and video monitoring.

Rodent Weanling to Adult Habitat - Centrifuge Facility Project Rodent Habitat. Provides food, water, waste management, environmental control, and video monitoring.

Avian Adult Habitat - Habitat to support adult birds. Provides food, water, environmental control, and video monitoring.

Large Aquatic Habitat - Large aquatic habitat (30-50 liters) that supports both fresh water and salt water organisms; provides environmental control (temperature, pH, gas levels, lighting), waste management, nutrient delivery and video monitoring.

Multi-discipline Equipment

Data Storage - Storage of data (video, microscopy, environmental sensor, etc.) collected from the habitats, the work area, etc.

Fluid Handling Tools - Tools for handling/transferring liquid solutions where automation is not provided including specimen samples (cell culture, blood, etc.), toxic fluids (fixatives), and infectious agents (viral containable). Does not include radioactive sample handling.

Compound Microscope - CCD microscope system for digital image analysis such as cell counting. Capabilities include high sensitivity for detecting changes in fluorescence, phosphorescence, and luminescence; phase contrast, differential interference phase contrast/polarization, dark field, and confocal optics; and video capability evolving from 2-d to 3-d. The system requires a tunable laser light source, filters, oil immersion lenses.

Work Area - Open and closed work areas to support manual operations including fixation, dissection, snap/quick freezing/ microscopy, etc.

Dissection Equipment - Plant and animal tissue dissection tools.

Dissecting Microscope - Low magnification (approximately 4X - 125X) stereo microscope with video capability. Used for specimen observations, dissections in the Glovebox.

Temperature Controlled Chamber - Refrigerator/Incubator Module with temperature control between 4°C and 37°C. *(With appropriate EUE, may accommodate multipurpose short duration experimentation such as cell and tissue cultures grown on semi-solid support media, seed germination, etc.)*

Temperature Controlled Laboratory Centrifuge - Laboratory centrifuge (distinct from 1-g control centrifuge) for sample processing (separation of suspended samples, blood components, etc.). Temperature controlled between 4°C and 37°C.

Radioisotope Handling Equipment - Equipment items for radioactive labeling and radioactive sample & solution storage.

Digital Multimeter - Portable multimeter (volt/amp meter) with adaptable inputs for measuring special or stand-alone sensors (such as leaf temperature sensors [thermocouples], pH, etc.).

Ion Selective Electrodes - Measures the concentration of specific ions including H⁺, Ca²⁺, K⁺, Cl⁻ in solution.

Spectrophotometer - Measures transmission or absorption of light through a solution to quantify protein concentration, nucleic acid concentration, turbidity, etc. Includes UV-Visible and fluorescence spectroscopy.

Mass Measurement Device- Device for measuring mass in microgravity.

Electrophysiology Measuring Equipment- Amplifiers, filters, electrodes, signal monitoring equipment.

Micromanipulation Device - Microscope-based device for single cell manipulations (such as positioning, cell fusion, microinjection).

Luminometer - Instrument to measure light emission/intensity.

Preservation and Storage Equipment

Snap/Quick Freezer - Device for instantly freezing samples. This is probably two different devices - one for thin sections, the other for large tissue samples.

-70°C Freezer - Freezer storage at -70°C to halt biochemical processes and prevent sample degradation.

Chemical Fixation Capability - Capability to chemically fix specimens whether or not fixation is automated.

Refrigeration (4°C) - Refrigerated storage for specimens, fixed samples, experimental solutions, etc.

-196°C Freezer - Freezer storage for samples that cannot be maintained at temperatures > -70°C.

-20°C Freezer - Freezer storage for samples that do not require ultra-cold storage temperatures.

Freeze Dryer - Device for freeze drying samples.

Ambient Storage - Room temperature storage for fixed samples, experimental solutions, etc.

HARDWARE CAPABILITIES MATRICES

Use of Matrices

The reference experiments are listed across the top of each matrix in a three letter shorthand notation, e.g. CA-a. The first two letters describe the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The final letter makes the notation unique by indicating where the experiment is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments.

Hardware items are identified along the side of each matrix. Checks in the matrix identify which items from the GBF hardware capabilities list are needed to support each experiment. Additional experiment unique equipment not provided by the Project may also be required.

The column labeled "Total" sums the checks along each row to give the number of experiments requesting each of the hardware capabilities listed.

To locate specific experiments, refer to the Experiment Index, page 2-1. This index lists the experiments in order, according to their experiment identification codes. To facilitate location, the experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page.

Critical Question Sub-categories

(C) Cell Biology

- (CA) Gravity Sensing/Response
- (CB) Transduction/Response
- (CC) Other

(D) Developmental Biology

- (DA) Early Developmental Events
- (DB) Later Developmental Events
- (DC) Parturition/Postnatal Growth
- (DD) Multigenerational Studies
- (DE) Behavior/Circadian Rhythms
- (DF) Aging
- (DG) Other

(P) Plant Biology

- (PA) Gravity Perception/Transduction/Response
- (PB) Reproduction/Development
- (PC) Metabolism/Transport
- (PD) Communities/Microecology

GBF CELL BIOLOGY EXPERIMENT HARDWARE CAPABILITIES

GBF HARDWARE CAPABILITIES		CA CA																									
TOTAL		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	a	b		
Habitats																											
Cell Culture Apparatus		33	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Rodent Weanling to Adult Habitat		1																						✓			
Multidiscipline Equipment																											
Data Storage		37	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Fluid Handling Tools		18	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Compound Microscope		10	✓																					✓			
Work Area		31	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Dissection Equipment		0																						✓			
Dissecting Microscope		0																									
Temp. Controlled Chamber*		9	(✓)	✓		✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)			
Temp. Controlled Lab Centrifuge		11				✓																					
Radioisotope Handling Equipment		4																									
Digital Multimeter		0																									
Ion Selective Electrodes		0																									
Spectrophotometer		6						✓	✓															✓			
Mass Measurement Device		0																									
Electrophysiology Measuring Equip.		0																									
Micromanipulation Device		0																									
Luminometer		0																									
Preservation and Storage Equipment																											
Snap/Quick Freezer		16				✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
-70°C Freezer		10																									
Chemical Fixation Capability		15		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
Refrigeration (4°C)		36	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
-196°C Freezer		16				✓																					
-20°C Freezer		3																									
Freeze Dryer		0																									
Ambient Storage		2																						✓			
* Parentheses are used to indicate experiments that can be performed in the Temperature Controlled Chamber given the appropriate Experiment Unique Equipment and in the absence of an on-orbit 1-g control.																											

* Parentheses are used to indicate experiments that can be performed in the Temperature Controlled Chamber given the appropriate Experiment Unique Equipment and in the absence of an on-orbit 1-g control.

[illegible]

* Parentheses are used to indicate experiments that are, at least in part, suited to the Temperature Controlled Chamber, given the appropriate Experiment Unique Equipment and no on-orbit 1-g control.

**GBF DEVELOPMENTAL BIOLOGY EXPERIMENT
HARDWARE CAPABILITIES, CONT.**

GBF HARDWARE CAPABILITIES	DC		DD		DD		DD		DD		DE		DE		DE		DE		DG		DG		DG		DG		
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Habitats																											
Cell Culture Apparatus*																											
Insect Habitat																											
Small Aquatic Habitat (Fresh Water)																											
Med. Aquatic Habitat (Fresh Water)																											
Avian Egg Incubator																											
Rodent Birthing Habitat																											
Small Aquatic Habitat (Salt Water)																											
Med. Aquatic Habitat (Salt Water)																											
Rodent Breeding Habitat																											
Avian Hatching Habitat																											
Rodent Rearing Habitat																											
Rodent Weaning to Adult Habitat																											
Avian Adult Habitat																											
Large Aquatic Habitat																											
Multidiscipline Equipment																											
Data Storage																											
Fluid Handling Tools																											
Compound Microscope																											
Work Area																											
Dissection Equipment																											
Dissecting Microscope																											
Temp. Controlled Chamber**																											
Temp. Controlled Lab. Centrifuge																											
Radioisotope Handling Equipment																											
Digital Multimeter																											
Ion Selective Electrodes																											
Spectrophotometer																											
Mass Measurement Device																											
Electrophysiology Measuring Equip.																											
Micromanipulation Device																											
Luminometer																											
Preservation & Storage Equip.																											
Snap/Quick Freezer																											
-70°C Freezer																											
Chemical Fixation Capability																											
Refrigeration (4°C)																											
-196°C Freezer																											
-20°C Freezer																											
Freeze Dryer																											
Ambient Storage																											

GBF PLANT BIOLOGY EXPERIMENT HARDWARE CAPABILITIES

[illegible]

Parenteses are used to indicate experiments that can be performed in the Temperature Controlled Chamber given the appropriate Experiment Unique Equipment and in the absence of an on-orbit 1-g control.

APPENDIX E

GBF EXPERIMENT DURATION MATRICES

<u>CONTENTS</u>	<u>PAGE</u>
• Description of Experiment Duration Categories	E-2
• Experiment Duration Matrices	
- Use of Matrices	E-3
- Cell Biology Experiment Duration Matrix	E-4
- Developmental Biology Experiment Duration Matrix	E-5
- Plant Biology Experiment Duration Matrix	E-7

DESCRIPTION OF EXPERIMENT DURATION CATEGORIES

The scientists were instructed to designate one or more of the flight opportunities described below for their reference experiments. The main differences in the flight opportunities that influenced each scientist's selection included experiment duration and available crew time. The experiment durations for Phase 1 (less than 2 weeks) and Phase 2 (1 month or greater) were clearly specified. Experiment durations for Phase 3 flights were to be specified by the scientists .

Flight opportunities

Phase 1: Utilization Flights (Spacelab class missions) - This phase provides flight opportunities similar to Spacelab missions with a total flight time of 16 days (approximately 11 days can be used for science payload operations on orbit). The Space Shuttle will be docked at the Space Station and members of the astronaut crew will be available to perform basic experiment procedures including specimen sampling and fixation for ground-based analysis.

Phase 2: Human Tended Capability (Spacelab class missions with extended unmanned durations) - This phase is similar to the Spacelab Class Missions except the science payload can be left unattended on board Space Station for 30 - 180 days after the Space Shuttle returns to Earth. This mission requires autonomous science experiments that do not require on-orbit crew manipulation.

Phase 3: Permanent Human Presence Capability (Space Station missions) - This is the most sophisticated stage of the Space Station in which the crew will be permanently on orbit and experiment durations are not limited. Research facility capabilities available on orbit will be more diverse and complex. Specimen handling and analysis will be able to be performed on-orbit.

Use of Matrices

The reference experiments are listed across the top of each matrix in a three letter shorthand notation, e.g. CA-a. The first two letters describe the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The final letter makes the notation unique by indicating where the experiment is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments.

Experiment durations are identified along the side of each matrix, grouped by flight opportunity. Checks in the matrix identify the experiments that fall into each duration category. Notice that some experiments are designed for more than one flight opportunity.

The column labeled "Total" sums the checks along each row to give the number of reference experiments fitting each of the experiment duration categories listed.

To locate specific experiments, refer to the Experiment Index, page 3-1. The Experiment Index lists the experiments in order, according to their experiment identification codes. To facilitate location, the experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page.

Critical Question Sub-categories

(C) Cell Biology

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- (DA) Early Developmental Events
- (DB) Later Developmental Events
- (DC) Parturition/Postnatal Growth
- (DD) Multigenerational Studies
- (DE) Behavior/Circadian Rhythms
- (DF) Aging
- (DG) Other

(P) Plant Biology

- (PA) Gravity Perception/Transduction/Response
- (PB) Reproduction/Development
- (PC) Metabolism/Transport
- (PD) Communities/Microecology

GBF CELL BIOLOGY EXPERIMENT DURATIONS

DURATION CATEGORY*	TOTAL	CA-a	CA-b	CA-c	CA-d	CA-e	CA-f	CA-g	CA-h	CA-i	CA-j	CA-k	CA-l	CA-m	CA-n	CA-o	CA-p	CA-q	CA-r	CA-s	CA-t	CA-u	CA-v	CA-w	CA-x	CA-y	CA-z
Phase 1: Utilization Flight																											
0-1 week (0-7 days)	2																										
1-2 weeks (8-16 days)	11			✓	✓																						
not specified	16	✓				✓																					
Phase 2: Human Tended Capability																											
1 month to 3 months	4	✓																									
greater than 3 months	1																										
not specified	2																										
Phase 3: Permanent Human Presence																											
1-2 weeks (8-16 days)	1																										
17 days to 1 month	3																										
1 month to 3 months	2					✓																					
greater than 3 months	1																										
not specified	1																										

* Parentheses identify experiments that were designated for more than one flight phase.

GBF DEVELOPMENTAL BIOLOGY EXPERIMENT DURATIONS

DURATION CATEGORY*	Phase 1: Utilization flight															
	DA-DA		DA-DA		DA-DA		DA-DA		DA-DA		DA-DA		DA-DA		DA-DA	
TOTAL	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
0-1 week (0-7 days)										✓						
1-2 weeks (8-16 days)			✓			✓							✓			
not specified	21	✓	✓	✓	✓		✓	(✓)	(✓)		✓	✓				
Phase 2: Human Tended Capability																
1 month to 3 months	5															
greater than 3 months	5												✓			
not specified	2												(✓)			
Phase 3 : Permanent Human Presence																
1-2 weeks (8-16)	0															
17 days to 1 month	1															
1 month to 3 months	4				✓											
greater than 3 months	10															
not specified	14						(✓)	(✓)		✓	✓	✓				

* Parentheses identify experiments that were designated for more than one flight phase.

GBF DEVELOPMENTAL BIOLOGY EXPERIMENT DURATIONS, CONT.

DURATION CATEGORY*		Phase 1: Utilization flight																	
		D8- D8- q r s	DC	DD-DD- a a b	DD-DD- c d e	DD-DD- f g h	DD-DD- i j k	DE- DE- a b c	DE- DE- d e f	DE- DE- g h i	DF- DF- a b c	DG-DG- d e f	DG-DG- g h i	DG-DG- j k l	DG-DG- m n o	DG-DG- p q r	DG-DG- s t u		
0-1 week (0-7 days)										√									
1-2 weeks (8-16 days)		√	√																
not specified														√	√	√	√	(√)	
Phase 2: Human Tended Capability																			
1 month to 3 months										√									
greater than 3 months														√				(√)	
not specified																		(√)	
Phase 3 : Permanent Human Presence																			
1-2 weeks (8-16)																			
17 days to 1 month										√									
1 month to 3 months									√										
greater than 3 months			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
not specified			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	(√)	
Parentphases identify experiments that were conducted during the specified duration category.																			

* Parentheses identify experiments that were

PLANT BIOLOGY EXPERIMENT DURATIONS

DURATION CATEGORY*	PA-PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA	PA
TOTAL	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
Phase 1: Utilization Flight																				
0-1 week (0-7 days)																				
1-2 weeks (8-16 days)	(✓)				✓															
not specified	19	✓	(✓)	✓	✓															
Phase 2: Human Tended Capability																				
1 month to 3 months	1																			
greater than 3 months	1																			
not specified	9	(✓)																		
Phase 3: Permanent Human Presence																				
1-2 weeks (8-16 days)	1				✓															
17 days to 1 month	1																			
1 month to 3 months	4																			
greater than 3 months	1				(✓)															
not specified	10																			

Parentheses identify experiments that were designated for more than one flight test.

APPENDIX F

GBF SPECIMEN TABLES

<u>CONTENTS</u>	<u>PAGE</u>
• Use of GBF Specimen Tables	F-2
• GBF Specimen Tables	
- Cell Biology Experimental Specimens	F-3
- Developmental Biology Experimental Specimens	F-5
- Plant Biology Experimental Specimens	F-7

Use of Specimen Tables

Specimens are listed in the tables by the scientific or common name(s) used in the reference experiments.

The experiments corresponding to each specimen type are identified in the tables by three letter codes, e.g. CA-a. The first two letters in the code indicate the discipline and the critical question sub-category that is the most directly addressed by the experiment, as detailed below. The third letter makes the notation unique by indicating where the experiment is ordered within its section. For example, experiment CA-a is the first of the Cell Biology Gravity Sensing/Response experiments. CA-b is the second of the Cell Biology Gravity Sensing/Response experiments, etc.

To locate specific experiments, refer to the Experiment Index, page 3-1. The Experiment Index lists the experiments in order, according to their experiment identification codes. To facilitate location, the experiment identification codes are also printed at the beginning of each reference experiment description and in the top right corner of each right hand experiment description page.

Critical Question Sub-categories

(C) Cell Biology

- (CA) Gravity Sensing/Response
- (CB) Transduction/Response
- (CC) Other

(D) Developmental Biology

- (DA) Early Developmental Events
- (DB) Later Developmental Events
- (DC) Parturition/Postnatal Growth
- (DD) Multigenerational Studies
- (DE) Behavior/Circadian Rhythms
- (DF) Aging
- (DG) Other

(P) Plant Biology

- (PA) Gravity Perception/Transduction/Response
- (PB) Reproduction/Development
- (PC) Metabolism/Transport
- (PD) Communities/Microecology

CELL BIOLOGY EXPERIMENTAL SPECIMENS

<u>Specimen type</u>	<u>Experiment(s)</u>
<u>Bacteria</u>	
<i>Bacillus subtilis</i>	
F:7	CA-a
FJ7	CA-a
F2b	CA-a
RHX	CA-a
<i>E. coli</i>	CA-m, CB-g, CB-i, CB-m
<i>Rhizobium</i>	CA-c, CA-d, CB-l
<i>Rhizobium leguminosarum</i> Biovar. <i>phaseoli</i> cells	PD-b
<u>Fungi</u>	
Yeast: <i>Saccharomyces cerevisiae</i>	CA-b, CB-f
<u>Insect cells</u>	
<i>Drosophila melanogaster</i> cell line	CB-r
<u>Mammalian cells/ cell lines</u>	
Colon ca cell line-human (LS174T)	CB-e
Epithelial cell line	CC-b
MDCK	CB-n
attachment-dependent	CB-a
Endothelial cell line	CB-b
Fibroblast cell line	CA-g, CC-b
3T3	CB-n, CB-p
3T3-L1 cells	CB-q
Dermal fibroblasts	CA-e
Leukemic cells-Human (HL-60)	CB-d
Lymphocyte cell lines	CB-a, CB-c, CB-k
Lymphocyte derived cells	CB-b
Cloned & short-term murine T lymphocytes	CB-h
Lymphoma cells- murine (S49)	CB-q
Macrophage cell lines (Human and murine)	CB-v
Myocytes	
Cardiac myocyte cultures	CB-j
Primary myocytes	CA-k
Neuroblastoma cell stock	CA-k
Mouse neuroblastoma cells	CB-d
Neuronal cultures	DB-e
Osteoblast and osteoclast cell lines	CB-u
Pheochromocytoma cell line (PC-12 cells)	CB-t
Skeletal muscle cell line (L8)	CA-k, CB-s

CELL BIOLOGY EXPERIMENTAL SPECIMENS CONTINUED...

<u>Specimen type</u>	<u>Experiment(s)</u>
<u>Plant cells</u>	
Algae (single cell, colonial, filamentary, other)	CC-a
<u>Solutions. Biological</u>	
Collagen	CA-h
Cytoplasmic fluid solution model	CA-j
Fibrinogen and thrombin solutions	CA-i
Intracellular fluid solution model	CA-j
<u>Tissues</u>	
Blood	CB-v
<u>Viruses</u>	
Vaccinia	CB-o
Sinbis	CA-l
Polio	CA-l
SV40	CA-l

DEVELOPMENTAL BIOLOGY EXPERIMENTAL SPECIMENS

<u>Specimen Type</u>	<u>Experiment(s)</u>
<u>Aquatic</u>	
Amphibians	DA-g, DB-g
Frogs	DB-d
<i>Ambystoma maculatum</i> (mole salamander)	DB-i
<i>Ambystoma mexicanum</i> (axolotl)	DB-i
<i>Cynops pyrrhogaster</i> (Japanese red bellied newt)	DB-a
<i>Rana pipiens</i> (terrestrial frog)	DB-i
<i>Xenopus laevis</i> (aquatic frog)	DA-h, DA-i, DB-f, DB-i
Marine Invertebrates	
<i>Aplysia californica</i> (marine mollusc)	DB-a
<i>Placopecten magellanicus</i> (sea scallop)	DG-b
Sea cucumbers	DA-l
Sea squirts	DA-l
Sea urchins	DA-b, DB-m
<i>Strongylocentrotus purpuratus</i>	DA-d, DB-n
<i>Lytechinus pictus</i>	DA-d
Scallops, shrimp or other food organism	DG-c
Starfish	
<i>Pisaster ochraceus</i>	DA-c, DA-e, DA-j
<i>Asterina miniata</i>	DA-j
<i>Tigriopus californicus</i> (marine copepod)	DE-b
Fresh water Invertebrates	
Pond snails	DA-b, DD-a
Plankton	DE-b
Microplankton representing three phyla	DG-a
<i>Isochrysis galbana</i> (T-ISO strain) (phytoplankton)	DG-b
<u>Avians</u>	
Chickens	DB-b, DB-c
White Leghorn	DB-h, DB-o, DB-p, DG-d
Quail	DE-e
Japanese Quail	DB-h
<i>Coturnix japonica</i>	DE-e
	DD-c
<u>Insects</u>	
<i>Drosophila melanogaster</i>	DE-c
	DA-i, DA-k, DA-p, DB-l, DB-s,
	DD-a, DD-b, DE-d, DF-a,
	DF-b
Gypsy moth	DA-a
Lepidopteran larvae: <i>Galleria mellonella</i>	DD-h
Silverfish	
<i>Ctenolepisma Longicaudata</i>	DA-o
<i>Lepisma saccharina</i>	DA-o
<i>Leucolepisma arenaria</i>	DA-o

DEVELOPMENTAL BIOLOGY EXPERIMENTAL SPECIMENS
CONTINUED...

<u>Specimen type</u>	<u>Experiment(s)</u>
<u>Nematodes</u>	
<i>C. elegans</i>	DA-n
<i>Steinernema carpocapsae</i>	DD-h
<i>Steinernema glaseri</i>	DD-h
<u>Rodents:</u>	
Hamsters, (Golden Syrian)	DA-m
Mice	DD-d
CD1 (ICR) outbred Swiss strain	DD-f, DD-g
B6CF1/An1 heterozygous mice	DG-f
mouse cell line	DG-h
Rats	DA-f, DB-j, DB-k, DB-q, DB-r, DC-a, DD-d, DD-e, DE-a, DE-c, CB-v, DG-g
Sherman rats	DG-e

PLANT BIOLOGY EXPERIMENTAL SPECIMENS

<u>Specimen type</u>	<u>Experiment(s)</u>
<u>Angiosperms</u>	PB-e, PB-o
Monocots	
Maize (<i>Zea mays</i>)	PA-e
Oat (<i>Avena</i>)	PC-d
Orchardgrass (<i>Dactylis glomerata</i> L.) leaf cultures	PB-k
Wheat (<i>Triticum</i>)	PB-m, PB-o, PB-p, PC-c, PC-g,
dwarf	PC-h
spike cultures	PB-r, PB-s, PC-a, PC-e
	PB-c, PB-j
Dicots	
<i>Arabidopsis thaliana</i>	PA-a, PA-d, PA-g, PB-a, PB-b,
	PB-i, PB-m, PB-r, PB-s, PB
	t
Bean (<i>Phaseolus vulgaris</i> L.)	PD-b
Brassica	
Rapid Cycling Brassica (<i>B.rapa</i>)	PB-a, PB-b, PB-i
dwarf	PC-a
<i>Haplopappus gracilis</i> cultured plantlets	PA-c
<i>Leucaena leucocephala</i>	PB-o, PB-m
Pea (<i>Pisum</i>), dwarf	PC-e
Soybean (<i>Glycine</i>)	PB-l, PC-a
Sweet Potato (<i>Ipomoea</i>)	PA-f
root explant cultures	PA-b
Tobacco (<i>Nicotiana</i>)	PA-a
Tomato (<i>Lycopersicon</i>)	PA-h
<u>Gymnosperms</u>	PB-e, PB-o
Canadian Yew (<i>Taxus canadensis</i>)	PC-b
Pine (<i>Pinus</i>)	PB-o, PB-m, PC-f
<u>Ferns</u>	
<i>Ceratopteris thalictroides</i>	PB-g
<u>Mosses</u>	
<i>Ceratodon</i> protonemal cultures	PB-f
<u>Algae</u>	
<i>Volvox</i>	PD-a
<i>Chlamydomonas</i>	PD-a
<u>Plant Cell and Tissue Cultures</u>	
Aseptic plant cell culture or organ tissue culture explant	PB-d, PB-h, PB-n
<i>Haplopappus gracilis</i> cultured plantlets	PA-c
Moss (<i>Ceratodon</i>) Protonemal cultures	PB-f
Orchardgrass (<i>Dactylis</i>) leaf segment cultures	PB-k
Sweet potato (<i>Ipomoea</i>) root explant cultures	PA-b
Wheat spike cultures	PB-c, PB-j

APPENDIX G

GBF AUTHOR INDEX

GBF Author Index

<u>Name</u>	<u>Experiment(s)</u>
1) Alpatov, Alexey M. 123007 Moscow Khoroshevskoye shosse 76-A	DE-c
2) Bergren, Todd BioServe Space Technologies University of Colorado Campus Box 429 Boulder, CO 80309	CA-h
3) Berry, Wallace D. Department of Physiology and Pharmacology University of Georgia Rm 710, Boyd GSRC Athens, GA 30602	DB-o, DB-p, DB-q, DB-r, DD-e
4) Birnboim, H. Chaim Ottawa Regional Cancer Centre 501 Smyth Road Ottawa, Ontario Canada K1H8L6	DG-h
5) Bloom, Arnold J. Department of Vegetable Crops University of California Davis, CA 95616	PA-h, PB-p, PC-c, PC-g
6) Braam, Janet Rice University Department of Biochemistry and Cell Biology P.O. Box 1892 Houston, TX 77251-1892	PA-d
7) Brown, Allan H. Gravitational Plant Physiology Laboratory University City Science Center 3401 Market St. Suite 350 Philadelphia, PA 19104-3323	PC-f
8) Brown, Christopher Manager, Plant Space Biology Mail Code BIO-3 Kennedy Space Center, FL 32899	PB-l, PC-h
9) Bubenheim, David MS 239-7 NASA Ames Research Center Moffett Field, CA 94035	PB-c, PC-a

GBF Author Index

<u>Name</u>	<u>Experiment(s)</u>
10) Capco, David G. Molecular and Cell Biology Program Department of Zoology Arizona State University Tempe, AZ 85287-1501	CB-q, DA-m
11) Carman, John G. Plants, Soils and Biometeorology Department Utah State University Logan, Utah 84322-4820	PB-j
12) Carper, Steve Science and Mathematics, UNLV 4505 South Maryland Pkwy Las Vegas, NV 89154-4001	CB-b
13) Cline, Morris G. Department of Plant Biology Ohio State University Columbus, OH 43210	PA-f
14) Conger, B.V. Department of Plant and Soil Science University of Tennessee Knoxville, TN 37901	PB-k
15) Crawford, Bruce J. Faculty of Medicine, Department of Anatomy University of British Columbia Vancouver, British Columbia V6T 1Z3 CANADA	DA-c, DA-e
16) Cseke, Leland J. Department of Biology The University of Michigan Natural Science Building Ann Arbor, MI 48109-1048	PC-b
17) Currie, R. William Department of Anatomy & Neurobiology Dalhousie University Halifax, Nova Scotia B3H 4H7 Canada	CB-j
18) Deitzer, Gerald F. Department of Horticulture University of Maryland College Park, MD 20742-5611	PA-g

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
19)	Dinsmore, Charles Department of Anatomy Rush Medical College 1653 West Congress Parkway Chicago, IL 60612	DB-g
20)	Dreschel, Thomas W. Plant Space Biology Mail Code BIO-3 Kennedy Space Center, FL 32899	PC-h
21)	Ellers, Olaf Department of Zoology University of California, Davis Davis, CA 95616	DB-m, DB-n
22)	Farmer, Ted Institute of Biological Chemistry Washington State University Pullman, WA 99164-6340	PB-t
23)	Feldman, Lewis Department of Plant Biology University of California Berkeley, CA 94720	PA-e
24)	Field, Christopher C. Southwest Missouri University Department of Biomedical Science 901 S. National Avenue Springfield, MO. 65804-0094	CA-l, CB-p
25)	Fortner, G. William Krug Life Sciences 1290 Hercules, Suite 120 Houston, TX 77058	CB-c
26)	Fritzsche, Bernd Department of Biomedical Sciences Division of Anatomy Creighton University Omaha, Nebraska 68178	DB-f
27)	Fuchs, Bruce A. Box 613 MCV Station Department of Pharmacology & Toxicology Medical College of Virginia/VCU Richmond, Virginia 23298-0613	CB-k

GBF Author Index

<u>Name</u>	<u>Experiment(s)</u>
28) Grymes, Rose NASA Ames Research Center MS 239-11 Moffett Field, CA 94035	CA-e, CB-o
29) Hayes, Dora K. U.S. Department of Agriculture ARS - BA - LPSI - LIL Beltsville, MD 20705-2350	DA-a
30) Hazel, Jeffrey R. Molecular and Cellular Biology Program Department of Zoology Arizona State University Tempe, AZ 85287-1501	CB-q
31) Hester, Patricia Y. Department of Animal Sciences Purdue University West Lafayette, IN 47907	DB-h, DE-e
32) Holley, D.C. Department of Biological Sciences San Jose State University 1 Washington Square San Jose, CA 95192-0100	DA-o
33) Holton, Emily M. Mail Stop 236-7 NASA Ames Research Center Moffett Field, CA 94035 (Green Book Experiment CH-E)	DB-j, DB-k, DC-a
34) Hughes, James P. Department of Life Sciences Indiana State University Terre Haute, IN 47809	CB-t
35) Johnson, Terry C. Division of Biology Kansas State University 234 Ackert Hall Manhattan, KS 66506	CB-d, CC-b
36) Jones, Timothy A. College of Dentistry U.M.N.C. Lincoln, NE 68583-0740	DB-b, DB-c

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
37)	Kaufman, Peter B. Department of Biology University of Michigan Natural Science Building Ann Arbor, MI 48109-1048	PC-b, PC-d
38)	Kaya, Harry Department of Nematology University of California Davis, CA 95616	DD-h
39)	Kearney, George P. Department Biosciences Walter Reed Army Institute of Research Washington, D. C. 20307-5100	CB-s
40)	Kessler, J.O. Physics Department, Building 81 University of Arizona Tucson, AZ 85721	CA-a, CC-a, PD-a
41)	Kinders, Robert J. Head, Binding Agents Development Molecular Diagnostics Research D907 Diagnostics Division, Abbott Laboratories North Chicago, IL 60064	CB-e
42)	Kirchen, Mary Los Angeles Orthopedic Hospital 2400 South Flower St. Los Angeles, CA 90060	CB-u
43)	Klaus, David M. BioServe Space Technologies University of Colorado Campus Box 429 Boulder, CO 80309	CB-i
44)	Krikorian, A.D. Department of Biochemistry and Cell Biology State University of New York Stony Brook, NY 11794	PB-d, PB-h
45)	Ledbetter, Mary Lee Department of Biology College of the Holy Cross One College Street, P. O. B Worcester, MA 01610	CB-n
46)	Levine, Howard G. Department of Biochemistry and Cell Biology State University of New York at Stony Brook Stony Brook, New York 11794-5215	PA-c

GBF Author Index

<u>Name</u>	<u>Experiment(s)</u>
47) Lewis, Marian L. Science Bldg., Room 360 University of Alabama Huntsville, AL 35899	CB-a
48) Lewis, Norman G. Institute of Biological Chemistry Washington State University 467 Clark Hall Pullman, WA 99164-6340	PB-e, PB-m, PB-o, PB-q
49) Lewis, Alan G. Department of Oceanography University of British Columbia Vancouver, British Columbia VAT 1Z4 Canada	DE-b
50) Marchin, George L. Division of Biology Kansas State University Manhattan, KS 66506	CB-g
51) Marshall, G. June Los Angeles Orthopedic Hospital 2400 South Flower St. Los Angeles, CA 90060	CB-u
52) Martindale, Valerie E. Clinical Investigation Directorate Wilford Hall Medical Center Lackland AFB San Antonio, TX 78236-5300	CA-f, CB-r
53) McGurl, Barry Institute of Biological Chemistry Washington State University Pullman, WA 99164-6340	PB-t
54) Meeker, Gabrielle NASA Ames Research Center MS T20G-2 Moffett Field, CA 94035	CA-d
55) Mendelson, N.H. Department of Molecular and Cellular Biology LSS Building University of Arizona Tucson, AZ 85721	CA-a

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
56)	Miller, Edwin S. Department of Microbiology and Immunology, School of Medicine University of Louisville Louisville, Kentucky 40292	CB-v
57)	Montelone, Beth A. Division of Biology Kansas State University 234 Ackert Hall Manhattan, KS 66506	CA-b, CB-f, CB-m
58)	Morrill, John Division of Natural Sciences 5700 N. Tamiami Trail Sarasota, FL 34234-2197	DA-b
59)	Mott, Glen Department of Pathology University of Texas Health Science Center 7703 Floyd Curl Drive San Antonio, TX 78284-7750	DA-f
60)	Murray, Joseph S. Division of Biology Kansas State University Manhattan, KS 66506	CB-h
61)	Musgrave, Mary E. Louisiana State University Agricultural Center Department of Plant Pathology and Crop Physiology Baton Rouge, LA 70803	PB-a, PB-b, PB-i
62)	Neff, Anton W. Medical Sciences Program Indiana University (Medicine) Bloomington, IN 47405	DA-h
63)	Nelson, Gregory A. Space Biological Sciences Group, MS 89-2 Jet Propulsion Laboratory 4800 Oak Grove Drive Pasadena, CA 91109	CA-m, DA-l, DA-n, DG-f
64)	O'Dor, Ronald K. Dalhousie University Department of Biology Halifax, Nova Scotia, CANADA B3H 4J1	DG-a, DG-b, DG-c

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
65)	Pass, Barry Faculty of Dentistry Dalhousie University Halifax, Nova Scotia Canada B3H 3J5	DG-g
66)	Pearce, Frederick J. Walter Reed Army Medical Center Walter Reed Army Institute of Research, Bldg. 40 Division of Surgery Washington, D.C. 20307-5100	CA-k
67)	Peck, Ernest J. Science and Mathematics, UNLV 4505 South Maryland Pkwy Las Vegas, NV 89154-4001	CB-b, DB-d, DB-e
68)	Phillips, Carey R. Department of Biology Bowdoin College Brunswick, ME 04011	CA-g, DA-g, DA-i, DD-a
69)	Piastuch, William C. Plant Space Biology Mail Code BIO-3 Kennedy Space Center, FL 32899	PB-r, PB-s
70)	Pollmann, Konrad BioServe Space Technologies University of Colorado Campus Box 429 Boulder, CO 80309	CA-i, CA-j
71)	Poovaiah, B.W. Department of Horticulture Washington State University Pullman, WA 99164-6414	PA-a
72)	Prentice, David A. Department of Life Sciences Indiana State University Terre Haute, IN 47809	CB-t
73)	Quebedeaux, Bruno Department of Horticulture University of Maryland College Park, MD 20742-5611	PC-e
74)	Raghavan, V. Department of Plant Biology Ohio State University Columbus, Ohio 43210	PB-g

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
75)	Sack, Fred D. Department of Plant Biology Ohio State University Columbus, Ohio 43210	PB-f
76)	Schatten, Gerald Department of Molecular Biology and Zoology University of Wisconsin 1117 W. Johnson Street Madison, WI 53706	DA-d
77)	Sonnenfeld, Gerald Department of Microbiology and Immunology, School of Medicine University of Louisville Louisville, Kentucky 40292	CB-v
78)	Stecker, R.E. Department of Biological Sciences San Jose State University 1 Washington Square San Jose, CA 95192-0100	DA-o
79)	Steele, Marianne K. Dept. of Physiology University of California San Francisco, CA 94143	DE-a
80)	Stricker, Stephen A. Department of Biology University of New Mexico Albuquerque, NM 87131	DA-j
81)	Sullivan, William T. Sinsheimer Lab Department of Biology University of California Santa Cruz, CA 95064	DA-p
82)	Swartz, Harry J. Department of Horticulture University of Maryland College Park, MD 20742	PA-b
83)	Takemoto, Larry Division of Biology Kansas State University 234 Ackert Hall Manhattan, KS 66506	CB-m

GBF Author Index

	<u>Name</u>	<u>Experiment(s)</u>
84)	Urban, James E. Division of Biology Kansas State University 234 Ackert Hall Manhattan, KS 66506	CA-c, CB-l
85)	Vasil, Indra K. Laboratory of Plant Cell and Molecular Biology Department of Vegetable Crops University of Florida Gainesville, Florida 32611-0514	PB-n
86)	Vasquez, Marcelo E. Eye Radiation & Environmental Research Laboratory, Columbia University 630 West 168th Street New York, NY 10032	DG-d
87)	Wassersug, Richard Department of Anatomy & Neurobiology Dalhousie University Sir Charles Tupper Bldg. Halifax, Nova Scotia Canada B3H 4H7	DB-i
88)	Wiederhold, Michael Department of OHNS University of Texas Health Science Center San Antonio, Texas 78284-7777	DB-a
89)	Wiley, Lynn Department of OB/GYN University of California School of Medicine Davis, CA 95616	DD-f, DD-g
90)	Wong, Peter Division of Biology Kansas State University 234 Ackert Hall Manhattan, KS 66506	PD-b
91)	Worgul, Basil Director, Eye Radiation & Environmental Research Laboratory, Columbia University 630 West 168th Street New York, NY 10032	DG-e

APPENDIX H

SSBRP SCIENCE WORKING GROUP MEMBERS

<u>CONTENTS</u>	<u>PAGE</u>
• Cell Biology Discipline Members	H-2
• Developmental Biology Discipline Members	H-3
• Plant Biology Discipline Members	H-4

Cell Biology SWG Members

Albright, Dr. Joseph F.
National Institute of Health
Chief, Basic Immunology Branch
Division of Allergy, Immunology & Transplantation
Solar Building, Room 4A25
Bethesda, MD 20882

Globus, Dr. Ruth K.
University of California at San Francisco
Dept. Med, Univ CA SF & V.A. Med. Ctr
San Francisco, CA 94121

Johnson, Dr. Terry
Kansas State University
Ackert Hall, Division of Biology, Rm 233
Manhattan, KS 66506

Krikorian, Dr. Abraham D.
State University of New York
Dept of Biochem & Cell Biol
Life Science Building
4th Flr, Rm 061
Stony Brook, NY 11794-5215

Sams, Dr. Clarence
NASA Johnson Space Center
Biomedical Operations & Research Branch
SD4, Build 37
Houston, TX 77058

Umminger, Dr. Bruce
Government
Division of Integrative Biology & Neuroscience
Room 685.13, National Science Foundation
4201 Wilson Blvd.
Arlington, VA 22230-0001

Developmental Biology SWG Members

Alberts, Dr. Jeffery
Indiana University
Dept of Psychology
Bloomington, IN 47405

Fineg, Dr. Jerry
University of Texas
Animal Resource Center
2701 Speedway
Austin, TX 78712

Fuller, Dr. Charles A.
University of California at Davis
Neurobiology, Physiology & Behavior
Davis, CA 95616

Hester, Dr. Patricia "Scotti"
Purdue University
Dept of Animal Sciences
1026 Poultry Science Building Rm 112
West Lafayette, IN 47907-1026

Katovich, Dr. Michael J.
University of Florida Health Science Center
Box 100487
College of Pharmacy
University of Florida, Gainesville, FL 32610

Packard, Dr. Mary "Kathy"
Colorado State University
Dept of Biology
Ft Collins, CO

Phillips, Dr. Carey
Bowdoin College
Department of Biology
Searles Building, Room 17
Brunswick, ME 04011

Wassersug, Dr. Richard
Dalhousie University
Division of Biology,
Ackert Hall, Rm 233
Manhattan, KS 66506

Wiley, Dr. Lynn Maxey
Reproductive Biology & Medicine
Dept of Obstetrics & Gynecology
University of California at Davis
Davis, CA 95616

Plant Biology SWG Members

Deitzer, Dr. Gerald
University of Maryland
Dept of Horticulture
2102 Holzapfel
College Park, MD 20742-5611

Feldman, Dr. Lewis
University of California at Berkeley
Dept of Plant Biology
111 Koshland Hall
Berkeley, CA 94720

Mitchell, Dr. Cary
Purdue University
Dept of Horticulture
West Lafayette, IN 47907

Sack, Dr. Fred
Ohio State University
Dept Botany
1735 Neil Avenue
Columbus, OH 43210

Scott, Dr. Tom
University of North Carolina
Dept of Biology
CG# 3280, Coker Hall
Chapel Hill, NC 27599

APPENDIX I

SSBRP SOLICITATION PACKAGE

<u>CONTENTS</u>	<u>PAGE</u>
• Cover Letter	I-2
• Description of Enclosures	I-3
• Experiment Categories:	
- Cell Biology	I-4
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• Reference Experiment Template	I-7
• Reference Experiment Example	I-8

Dear Colleague,

You have been recommended by one of your colleagues, on the basis of the nature and quality of your research, as an individual who could contribute to our understanding of how life is affected by the environmental conditions of Space. Towards this objective, we are requesting your expertise in developing one or more "Life Science Reference Experiments" that could be conducted in the unique environment of space. Life Science Reference Experiments are one page summaries that describe the hypothesis, scientific rationale, general approach, and equipment needed for the experiment.

One of the hardware items that will be used for biological research on the International Space Station is an Egg Incubator. What is envisioned is a "Space Incubator" capable of housing avian and reptilian eggs up to (but not necessarily including) hatching. We are specifically interested in obtaining Reference Experiments that make use of this piece of equipment.

Beyond that, there are no restrictions on the type of experiments that can be outlined for a Reference Experiment. Such experiments, for example, could be related to your on-going research, or something totally new and different. Reference Experiments can be for any research that you think would answer a basic question in biology, using avian or reptilian eggs in microgravity. The equipment needs, beyond the Egg Incubator itself, should be specified in terms of generally available laboratory equipment. A Reference Experiment template and other information is enclosed to aid you in the development of your Reference Experiments.

If you cannot for whatever reason provide a Reference Experiment, could you suggest the names of other scientists whom you feel might be able to submit a Reference Experiment?

Please understand that NASA is not at this time soliciting experiments for flight on the International Space Station. But NASA does need Reference Experiments to aid in designing hardware and planning for those future space flights. The Reference Experiments, which you and others submit, will be compiled and published as a NASA document that will be made available to the public. Submitting a Reference Experiment does not mean that NASA is obligated to providing a spaceflight opportunity for the Reference Experiment. In other words, this is not a NASA Research Announcement (NRA) or Announcement of Opportunity (AO). However, we would be happy to add your name to the mailing list to receive future NRAs and AOs in Space Life Science.

2

Previous experience has shown that acceptable Reference Experiments can be prepared in as little as 20 minutes. We hope that you can take that time to help us out. Thank you for your time and effort. The contribution of your expertise will greatly enhance the quality and functionality of the Egg Incubator and ultimately the quality of the science that can be accomplished on the Space Station.

Sincerely,

Catherine Johnson
Space Station Biological Research Project

DESCRIPTION OF ENCLOSURES

1. **NASA Facts:** Information on the Gravitational Biology and Centrifuge Facilities.
2. **Experiment Categories:** Reference experiments should focus on the critical space biology research issues for Developmental Biology as indicated on the Experiment Categories enclosure. These areas are being defined quite broadly to include developmental processes at all levels of the biological hierarchy, and covering systems such as the vestibular, cardiovascular, and nervous.
3. **Example Reference Experiments:** An example of a reference experiments is enclosed to show the desired detail and content of the reference experiments.
4. **Reference Experiment Template:** Reference experiments should be written in the format of the enclosed "Reference Experiment Template". If you would like to work with an electronic copy of this template, please contact Karolyn Ronzano at karolyn_ronzano@qmgate.arc.nasa.gov and request a soft-copy of this template. Copies of the template may be made if you wish to prepare more than one reference experiment, or if a colleague of yours would also like to participate.

We would like to receive your contribution by September 30, 1995. However, if your schedule requires additional time, we are happy to receive your input after this date as well.

EXPERIMENT CATEGORIES

Discipline:

A. Cell Biology

1. Cell Activation - (Gravity perception; interactions with cell and environment)
 - Cell-cell interactions, cell-surface interactions
 - Activation sequences
 - Agonist-ligand interactions
2. Transduction - (Intracellular events)
 - Second messenger pathways
 - Genetic control
3. Growth, Differentiation, Dedifferentiation
 - Protein synthesis
 - Post translational modifications
4. Metabolism
 - Mitochondria
 - Aerobic
 - Anerobic
5. Functional Capacity
 - Muscle contraction
 - Phagocytosis
 - Antibody production
6. Stress Response
 - Ubiquitin pathway
 - Heat-shock proteins
 - Acclimation and compensation mechanisms
7. Other Considerations
 - Eukaryotic vs. prokaryotic organisms

EXPERIMENT CATEGORIES

Discipline:

B. Developmental Biology

1. Role of gravity in developmental biology
 - Alterations in the developmental ontogeny of animals raised in space vs. animals raised in 1 g
 - Critical windows of susceptibility for developmental processes
 - Reversal of gravity related effects
 - Gravity-induced dys-synchrony during embryonic development
2. One g dependent developmental mechanisms
3. Gravity effects on early developmental events
 - Gametogenesis, early germ cell maturation
 - Fertilization
 - Embryonic axis determination
 - Zygotic gene activation
4. Effects on developmental phases across different species and phyla.
 - Aquatic vs. terrestrial animals
5. Stages of perturbations of biological rhythms
6. Organogenesis and development of anatomical structures
7. Structural and morphometric alterations
8. Gravity effects on behavior
 - Parent/offspring interactions
 - Sexual behavior
9. Sexual maturation
10. Reproductive systems
11. Effects of gravity on lifespan de-limiters
12. Joint effects of radiation and microgravity

EXPERIMENT CATEGORIES

Discipline:

C. Plant Biology

1. Gravity perception, transduction, and response mechanisms
 - Gravity perception mechanisms
 - Sequential events in gravity transduction and response
 - Gravity sensing by a single cell
 - Effective gravity thresholds
 - Changes in the routes of gravity perception, transduction, and response
 - Perception and response differences between species.
2. Reproduction and development processes
 - Reproduction through more than one generation
 - Cell division
 - Cell, tissue, and organ differentiation
 - Life cycle stages (embryogenesis through senescence)
 - Competency of microgravity grown tissues and organs
 - Growth rates
 - Adaptation to microgravity
 - Developmental effects due to unique interactions of space radiation and microgravity (or other environmental factors)
3. Metabolism, photosynthesis, and transport processes
 - Changes in photosynthetic apparatus, anabolic and catabolic pathways
 - Synthesis and storage of support polymers
 - Effect on membranes and transport during uptake and secretion
 - Nutrient absorption pathways
 - Long distance water transport and transpiration
 - Mechanisms for polarization of transport systems

Reference Experiment Template

1. Discipline

2. **Author**(*Responsible Science Working Group member: name*
Author: name, address, phone number)

3. Experiment Title

4. Purpose/Hypothesis (~ 3 sentences)

5. Scientific Rationale/Rationale for Microgravity (~ 4 sentences)

6. Approach

A. FLIGHT/EXPERIMENT DURATION

B. GENERAL APPROACH DESCRIPTION: (~ 4 sentences)

C. NUMBER AND TYPE OF SPECIMEN:

D. MEASUREMENTS/SAMPLE HANDLING: (*brief descriptions*)

Preflight:

In-flight:

Postflight:

E. SPECIFIC SAMPLE ANALYSIS: (*brief descriptions*)

In-flight:

Postflight

F. EXPERIMENT CONTROLS: (*brief descriptions*)

In-flight:

Ground-based:

7. Hardware Requirements

A. MINIMUM ON ORBIT REQUIREMENTS

B. HARDWARE REQUIREMENTS FOR GROUND

8. *Names, addresses, and phone numbers of other scientists interested in contributing:*

EXAMPLE -REFERENCE EXPERIMENT

1. Discipline: Developmental Biology

2. Author/Reference: (*Author: name, address, phone number*)

Dr. Wallace D. Berry
Department of Physiology and Pharmacology
University of Georgia
Rm 710, Boyd GSRC
Athens, GA 30602

3. Experiment Title: Development of Avian Vitamin D Endocrine System in Microgravity: Vitamin D Metabolites and Mineral Homeostatic Hormones

4. Purpose/Hypothesis: (*~ 3 sentences*)

Microgravity will cause changes in relative levels of vitamin D metabolites, parathyroid hormone, and calcitonin.

5. Scientific Rationale/Rationale for Microgravity: (*~ 4 sentences*)

Adaptation to microgravity drives changes in bone physiology that result in bone mineral loss/redistribution and negative calcium balance. Corresponding changes should occur in mineral homeostatic hormones. These hormones are important in the function of a wide range of tissues. Therefore, the information gained in this study will be valuable in determining the mechanisms of microgravity effects and in anticipating long term effects.

6. Approach:

A. Experiment Duration: (*1-90 days*)

7-16 days

B. General Approach Description: (*~ 4 sentences*)

Fertile chicken eggs will be incubated in-flight. Plasma or serum will be collected from embryos or chicks postflight and frozen (-70° or -196°C). Vitamin D metabolites, parathyroid hormone, and calcitonin will be assessed postflight.

C. Number and Type of Specimen: Four samples drawn from a minimum of four flight eggs (16 samples). Additional samples if 1-g centrifuge is available.

D. Measurements/Sample Handling: (*brief descriptions*)

Preflight: None

In-flight: None

Postflight: Blood draws, blood processing, sample freezing. (Blood draw equipment and sample vials, centrifuge for processing plasma or serum, and freezer (-70C or -196C) required.)

E. Specific Sample Analysis: (*brief descriptions*)

In-flight: None.

Postflight: Assay of vitamin D metabolites, PTH, CT.

F. Experiment Controls: *(brief descriptions)*

In-flight: 1-g centrifuge controls if centrifuge available, otherwise none.

Ground-based: Controls will parallel flight experiments.

APPENDIX J

SSBRP HARDWARE CAPABILITIES LIST

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SSBRP HARDWARE CAPABILITIES LIST

Habitats (Both 0g and 1g environments provided on-orbit)

Advanced Animal Habitat-Centrifuge
Aquatic Habitat
Cell Culture Unit
Egg Incubator
Insect Habitat
Mouse Development Insert
Plant Research Unit

Laboratory Support Equipment

Battery Charger
Camera Locker
Cleaning Equipment
Compound Microscope
Digital Multimeter
Digital Recording Oscilloscope
Dissecting Microscope (Stereo Macrocope)
Dissection Equipment
EM-Shielded Locker
Film Locker
Fluid Handling Tools
Freezer (-20° C)
Freezer (-80° C)
General Purpose Hand Tools
Incubator
Light Meter
Micro Mass Measurement Device
Passive Dosimeter
pH/Ion Specific Electrode Meter
Quick/Snap Freezer (-196° C)
Refrigerated Centrifuge
Refrigerator (4° C)
Slow Freeze Accessories
Small-Mass Measuring Device
Specimen Labeling Tools
Still Cameras
Storage Freezer (-196° C)
Video Cameras

SSBRP HARDWARE CAPABILITIES DEFINITIONS

Habitats (Both 0g and 1g environments provided on-orbit)

Advanced Animal Habitat-Centrifuge - Habitat to accommodate adult (post-weanling) mice and rats. Provides food, water, waste management, environmental control, and video monitoring.

Aquatic Habitat - Six replicate specimen chambers are to be provided, each with its own independent biofilter and water quality management. Environmental control of temperature, oxygen, pH, and photoperiod will be available. Video monitoring, specimen access, and long-term food delivery to all life stages will also be provided. The accommodation of both freshwater and marine organisms is expected, as is some flexibility in specimen chamber configuration.

Cell Culture Unit - The cell culture unit provides life support and experiment capability for animal, microbial, and plant suspension cultures, attachment cultures, tissues less than 4 mm in length, and non-feeding aquatic specimens less than 200 μm in length. Automatic delivery of additives including fixatives, temperature control, gas mixture infusion into the media, and on-orbit variable gravity conditions will be accommodated in the design. Data storage, downlinking, and command capability will also be included.

Egg Incubator - Environmentally controlled incubator (temperature, humidity, O_2 , CO_2) to maintain avian and reptilian eggs. Internal centrifuge with selectable gravity levels, auto fixation, and egg turning capability.

Insect Habitat - Habitat to maintain the fruit fly, *Drosophila melanogaster*, and possibly other insects. Provides environmental control of atmosphere, humidity, lighting, and temperature. The habitat features separation of adults from offspring through successive generations, video monitoring, and centrifugation for gravity control.

Mouse Development Insert - Specimen cage insert to the AAH-C to accommodate female mice during pregnancy and birth, and their neonates from birth through weaning. The MDI should facilitate nursing and huddling of the neonates, and dam-neonate interaction. Provides food, water, waste management, environmental control, lighting, and video monitoring.

Plant Research Unit - Plant growth chamber with approximately 550 cm^2 growing area. Provides environmental monitoring and control (temperature, relative humidity, CO_2 levels and lighting) Modular construction will allow choice of nutrient delivery systems and substrates, and fluorescent or LED illumination systems. Light levels of up to 650 $\mu\text{Moles.m}^{-2}.\text{s}^{-1}$ (fluorescent) or 1000 $\mu\text{Moles.m}^{-2}.\text{s}^{-1}$ (LED) will be available

Laboratory Support Equipment

Battery Charger - A compact device for charging nickel-cadmium batteries that are used by a number of small instruments (e.g., oscilloscopes, multimeters). Batteries of the following sizes can be recharged: D, C, AA, AAA (nominal 1.2 Vdc) and 9 Vdc.

Camera Locker - The Camera Locker provides the equipment storage space for all LSE cameras and associated components, including lights, mounting hardware, etc.

Cleaning Equipment - Tools and supplies necessary to perform housekeeping and cleanup of laboratory equipment, including the interior surfaces of gloveboxes.

Compound Microscope - Standard bench top microscope with objective magnifications up to 100X and Kohler illumination to support phase contrast microscopy for cellular and sub cellular observations. It includes accessories for interference phase contrast microscopy, fluorescence, video and still photography

Digital Multimeter - A general purpose hand-held voltage, current, and resistance meter for use in maintenance and adjustment of electrical equipment, and for temporary use in experiments.

Digital Recording Oscilloscope - A hand-held, portable instrument for detecting, digitizing, recording, and displaying periodic and transient electrical waveforms for use in electronic maintenance and experiment set-up calibration.

Dissecting Microscope (Stereo Macroscop) - A system which provides the capability for microscope aided inspections and operations within the confines of a glovebox. It uses long working distance optics to allow for operations such as specimen dissection. It has the capability to provide for incident and transmitted lighting, and still and video photography.

Dissection Equipment - The Dissection Equipment is a set of generic tools that facilitate biological specimen dissection and manipulation: forceps, scalpels, probes, clamps, etc., and a micromanipulator for the dissecting microscope.

EM-Shielded Locker - The EM-Shielded Locker will provide general purpose storage of exposed and unexposed magnetic media to shield the contents from electromagnetic and ionizing radiation.

Film Locker - The Film Locker will provide the capability for general purpose storage of exposed and unexposed film and shielding of the contents from environmental radiation.

Fluid Handling Tools - The Fluid Handling Tools are devices designed to mix, transfer, and measure volumes of liquids in microgravity, including hand tools such as syringes, squeeze bulbs, disposable droppers, pumps, a hand spin centrifuge for bubble removal, vacuum cleaners, tubing, pipettes, disposable and reusable containers, and chemical fixation tools.

Freezer (-20° C) - The -20° C Freezer is a low temperature storage facility used to preserve experiment specimens and supplies until they can be used on-orbit or analyzed on the ground.

Freezer (-80° C) - The -80° C Freezer is a low temperature storage facility used to preserve experiment specimens, samples, and supplies until they can be used on-orbit or analyzed on the ground.

General Purpose Hand Tools - A set of mechanical and battery-operated hand tools for integration, de-integration, operating, maintaining, and repairing experiment facilities and laboratory support equipment. It will also include tools for micro-operations, and tools for soldering and wire wrapping.

Incubator - The Incubator is a controlled environmental chamber for growing cell and tissue cultures.

Light Meter - Meter to measure light levels in habitats

Micro Mass Measurement Device - The Micro Mass Measuring Device is an instrument that can accurately determine the mass of solid chemicals, liquids, tissues, samples, organs, etc., in the range of 1 mg to 10 gm.

Passive Dosimeter - The Passive Dosimeter consists of a Thin Layer Dosimetry (TLD) type badge reader/annealer to determine badge exposure to radiation and passive badges to accumulate dosages of radiation.

pH/Ion Specific Electrode Meter - The pH/Ion Specific Meter is a high impedance meter which measures the concentration of hydrogen ions (pH) or specific cations/anions in a liquid solution

Quick/Snap Freezer (-196° C) - The -196° C Quick/Snap Freezer is a portable -196° C freezer capable of vitrifying small tissue samples and quick freezing medium sized contained samples while being operated in the Life Science Glovebox.

Refrigerated Centrifuge - Device which uses centrifugal acceleration to separate materials by density in a temperature controlled environment.

Refrigerator (4° C) - The 4° C Refrigerator is a low temperature storage freezer used to preserve experiment specimens and supplies.

Slow Freeze Accessories - The Slow Freeze Accessories are containers that facilitate cooling 5 ml vials of biological samples at 1° C per minute from ambient to -80°C, then quickly to -196° C. These containers are used in conjunction with the -80° C Freezer and -196° C Storage Freezer.

Small-Mass Measuring Device - An instrument that can accurately determine the masses of solid, semi-solid, and liquid materials (including live specimens) in the range of 1 gm to 5000 gms, in microgravity.

Specimen Labeling Tools - The Specimen Labeling Tools are a dispenser and bar-coded, self-adhesive labels for attachment to specimen containers.

Still Cameras - 35 mm still cameras and accessories for general laboratory photography.

Storage Freezer (-196° C) - The -196° C Storage Freezer provides for storage and retrieval of supplies, samples and specimens below -183° C.

Video Cameras - Video cameras and accessories for general laboratory photography.

APPENDIX K

SSBRP AUTHOR INDEX

SSBRP Author Index

<u>Name</u>	<u>Experiment(s)</u>
1) Bast, Klaas Kramer Aalt Vrije Universiteit Dept. of Pharmacochimistry de Boelelaan 1083 1081 HV Amsterdam The Netherlands	DB-d(2)
2) Beckage, Nancy E. Department of Entomology 5419 Boyce Hall University of California at Riverside Riverside, CA 92521-0314	DB-l(2)
3) Brown, John J. Department of Entomology Washington State University Pullman, WA 99164-6382	DA-i(2)
4) Burghardt, Gordon M. Department of Psychology University of Tennessee Knoxville, TN 37996-0900	DE-d(2)
5) Burton, Ronald S. Marine Biology Research Division Scripps Institution of Oceanography University of California, San Diego LaJolla, CA 92093-0202	DD-a(2)
6) Cheng, Keith C. Division of Experimental Pathology Penn State College of Medicine 500 University Dr. Hershey, PA 17033	DG-d(2), DG-e(2), DG-f(2)
7) Ciereszko, Andrzej School of Natural Resources The Ohio State University 2021 Coffey Road Columbus, OH 43210	DA-f(2)
8) Conrad, Gary W. Division of Biology-Ackert Hall Kansas State University Manhattan, KS 66506-4901	DB-q(2)
9) Crawford, Bruce J. Faculty of Medicine/Department of Anatomy University of British Columbia Vancouver, British Columbia V6T 1Z3 Canada	DA-b(2)

SSBRP Author Index

<u>Name</u>	<u>Experiment(s)</u>
10) Dabrowski, Konrad School of Natural Resources The Ohio State University 2021 Coffey Road Columbus, OH 43210	DA-e(2), DA-f(2), DC-e(2), DC-f(2), DC-i(2)
11) Diana, James S. School of Natural Resources and Environment University of Michigan Ann Arbor, MI 48109-1115	DC-b(2)
12) Doty, Stephen B. R 424 Research Building The Hospital for Special Surgery 535 E. 70th Street New York, NY 10021	DB-v(2)
13) Edmands, Suzanne Marine Biology Research Division Scripps Institution of Oceanography University of California, San Diego LaJolla, CA 92093-0202	DD-a(2)
14) Eyal-Giladi, Hefzibah Department of Cell and Animal Biology Hebrew University Jerusalem 91904 Israel	DA-m(2)
15) Farrell, A.P. Biological Sciences Simon Fraser University Burnaby, B.C. V5A 1S6 Canada	DG-c(2)
16) Fermin, Cesar D. Department of Pathology and Lab Medicine 1430 Tulane Ave./SL 79 Tulane Medical School New Orleans, LA 70112-2699	DB-p(2)
17) Gibbons, J. Whitfield Savannah River Ecology Laboratory University of Georgia P.O. Drawer E Aiken, SC 29802	DA-l(2)
18) Greenstock, Clive L. AECL Radiation Biology & Health Physics Branch Chalk River, ON Canada KOJ 1J0	CB-a(2), DD-b(2)

SSBRP Author Index

<u>Name</u>	<u>Experiment(s)</u>
19) Gupta, Ayodhya P. Department of Entomology J.B. Smith Hall, Cook College Rutgers University New Brunswick, NJ 08903-0231	CA-a(2), CB-b(2)
20) Hackett, Perry B. Department of Genetics and Cell Biology University of Minnesota 1445 Gortner ave. St. Paul, MN 55108-1095	DA-g(2)
21) Ho, Robert K. Department of Molecular Biology Princeton University Princeton, NJ 08544	DA-a(2)
22) Horn, Eberhard University of Ulm, Department of Neurology Section of Neurophysiology Albert-Einstein-Allee 11 D-89081 Ulm, Germany	DA-h(2), DG-b(2)
23) Icardo, Jose M. Department of Anatomy and Cell Biology University of Cantabria 39011-Santander Spain	DB-s(2)
24) Ishay, Jacob S. Department of Physiology & Pharmacology Sackler Facility of Medicine Tel-Aviv University Israel	DB-g(2)
25) Janzen II, Fredric J. Department of Zoology and Genetics Iowa State University Ames, IA 50011	DA-j(2), DB-w(2)
26) Kam, Zvi Molecular Cell Biology Weizmann Institute of Science REHOVOT 76100 Israel	DA-d(2)
27) Kerschmann, Russell L. University of California at San Francisco Department of Pathology, Building 3 San Francisco General Hospital San Francisco, CA 94110	DB-r(2)

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<u>Name</u>	<u>Experiment(s)</u>
28) Lin, Feng School of Natural Resources The Ohio State University 2021 Coffey Road Columbus, OH 43210	DA-e(2), DC-f(2)
29) Malacinski, George M. Department of Biology Indiana University Bloomington, IN 47405	DA-c(2)
30) Malouvier, Alexandre NASA Ames Research Center Mail Stop 236-7 Moffett Field, CA 94035-1000	DB-a(2)
31) Morey-Holton, Emily NASA Ames Research Center Mail Stop 236-7 Moffett Field, CA 94035-1000	DB-a(2)
32) Neff, Anton W. Medical Sciences Program Indiana University Bloomington, IN 47405	DA-c(2)
33) Nemeth, Richard S. Department of Zoology University of New Hampshire Durham, NH 03824	DC-a(2)
34) Ramaswamy, Sonny Department of Entomology & Plant Pathology Mississippi State University, MS 39762-9775	DB-m(2)
35) Reed, Darcy A. Department of Entomology 5419 Boyce Hall University of California at Riverside Riverside, CA 92521-0314	DB-l(2)
36) Rifkind, Arleen B. Cornell University Medical College 1300 York Ave. New York, NY 10021	DB-o(2)
37) Ross, Muriel D. NASA Ames Research Center Mail Stop 239-11 Moffett Field, CA 94035-1000	DC-j(2)

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<u>Name</u>	<u>Experiment(s)</u>
38) Steiner, William W. M. Pacific Islands Science Center Rm 406, Gilmore Hall--UH 3050 Maile Way Honolulu, HI 96822	DD-c(2)
39) Steyger, P.S. R.S. Dow Neurological Sciences Institute Legacy Good Samaritan Hospital and Medical Center 1120 NW 20th Avenue Portland, Oregon 97209	DB-i(2), DB-j(2), DB-k(2)
40) Taub, Frieda B. School of Fisheries University of Washington Box 355100 Seattle, WA 98195	DB-h(2), DC-g(2), DG-g(2)
41) Turner, Bruce J. Department of Biology Virginia Polytechnic Institute and State University Blacksburg, VA 24061-0406	DC-h(2)
42) van Twest, Jacqui The Bionetics Corporation Mail Code BIO-3 Kennedy Space Center, FL 33899	DB-n(2), DB-t(2)
43) Vo-Dinh, Tuan Advanced Monitoring Development Group Oak Ridge National Laboratory P.O. Box 2008 Oak Ridge , TN 37831-6101	DB-c(2)
44) Webb, Paul W. School of Natural Resources and Environment University of Michigan Ann Arbor, MI 48109-1115	DE-c(2)
45) Weber, Daniel Marine and Freshwater Biomedical Sciences Center University of Wisconsin 600 E. Greenfield Ave. Milwaukee, WI 53204	DC-d(2), DE-a(2), DE-b(2)
46) Wentworth, Bernard C. Department of Poultry Science University of Wisconsin Madison, WI 53706-1284	DA-k(2), DG-h(2), DG-i(2), DG-j(2)

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<u>Name</u>	<u>Experiment(s)</u>
47) Whittow, G. Causey Department of Physiology John A. Burns School of Medicine University of Hawaii 1960 East West Road Honolulu, HI 96822	DB-u(2)
48) Wiederhold, Michael L. Dept. Otolaryngology- Head & Neck Surgery University Texas Health Science Center 7703 Floyd Curl Drive San Antonio, TX 78284-7777	DC-c(2)
49) Winn, Richard N. Center for Applied Isotope Studies University of Georgia 120 Riverbend Road Athens, GA 30606	DG-a(2)

APPENDIX L

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13. ABSTRACT (Maximum 200 words) The Space Station Biological Research Project (SSBRP), which is the combined efforts of the Centrifuge Facility (CF) and the Gravitational Biology Facility (GBF), is responsible for the development of life sciences hardware to be used on the International Space Station to support cell, developmental, and plant biology research. The SSBRP Reference Experiment Book was developed to use as a tool for guiding this development effort. The reference experiments characterize the research interests of the international scientific community and serve to identify the hardware capabilities and support equipment needed to support such research. The reference experiments also serve as a tool for understanding the operational aspects of conducting research on board the Space Station. This material was generated by the science community by way of their responses to reference experiment solicitation packages sent to them by SSBRP scientists. The solicitation process was executed in two phases. The first phase was completed in February of 1992 and the second phase completed in November of 1995. Representing these phases, the document is subdivided into a Section I and a Section II. The reference experiments contained in this document are only representative microgravity experiments. They are not intended to define actual flight experiments. Ground and flight experiments will be selected through the formal NASA Research Announcement (NRA) and Announcement of Opportunity (AO) experiment solicitation, review, and selection process.				
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